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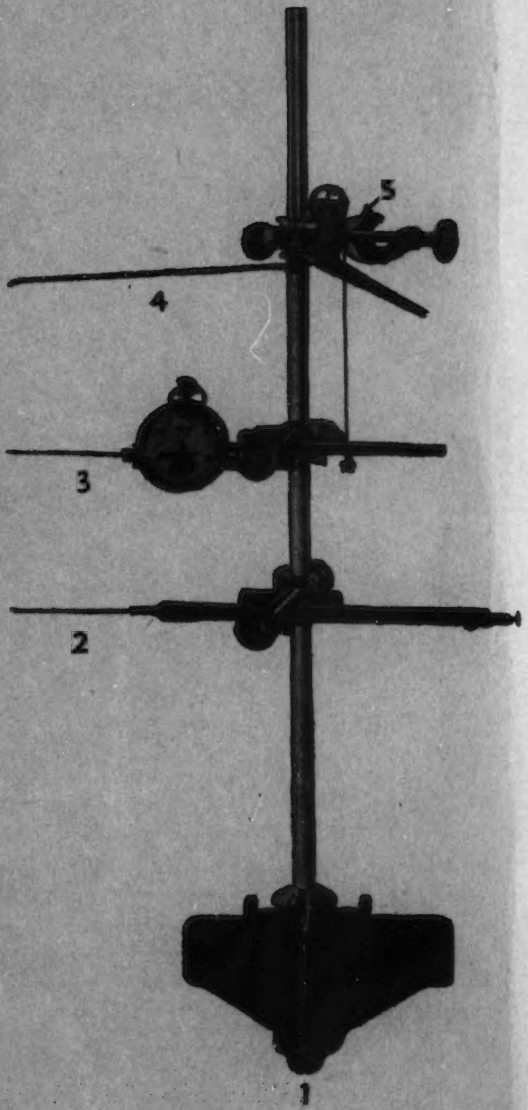

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THE EFFECT OF THE PASTEURIZATION OF MILK ON THE UTILIZATION OF ITS CALCIUM FOR GROWTH IN THE RAT

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It seems unreasonable to expect that the moderate heating of cow's milk involved in its pasteurization would profoundly affect its nutritive value, except in the direction of a more or less complete destruction of its content of vitamin C (1), (2), (3). However, other deleterious effects have been reported in the literature. In 1920 Daniels and Loughlin (4) obtained indications from feeding experiments with rats that the pasteurization of milk caused a diminution of its nutritive value, an effect which was explained as due to the precipitation and loss of part of its calcium, which adhered to the walls of the pasteurizing vessel. Four years later, in much better controlled experiments with young children, Daniels and Stearns (5) found that the calcium and phosphorus of pasteurized milk are distinctly less well utilized than the calcium and phosphorus of milk sterilized by raising it quickly to the boiling point and maintaining this temperature for only a few minutes. While one may question the full significance of balance periods of only 3 days' duration, the consistency observed among the results of these experiments speaks strongly in favor of the conclusion drawn. Presumably, at least as distinct a difference would have been obtained under the conditions chosen had the comparison been made between pasteurized and raw milk. Willard and Blunt (6) found much less distinct and less consistent differences in the utilization of the calcium and phosphorus of pasteurized and evaporated milk with children, while with adults no differences at all were observed. In 1926, Magee and Harvey (7) compared pasteurized and raw milk in balance experiments with two young pigs. Although it was concluded that the pasteurized milk promoted less complete retention of both calcium and phosphorus than the raw milk, the extreme variation existing among the 48 hour results ob-

tained during the first period of the experiment, during which pasteurized milk was fed to one pig and raw milk to the other, removes all possibility of accurate comparison. The latest contribution to this problem is that of Krauss, Erb, and Washburn (8), who, using the paired-feeding method with rats, detected no difference, either in the growth-promoting value or in the calcifying power of raw and pasteurized milk. In these experiments exclusive milk feeding was employed.

PLAN OF THE EXPERIMENT. This review of the literature seemed to the authors to warrant further investigation. However, another contribution in this field would be hard to justify unless it represents something new in method and involves adequate control of all the gross factors concerned in calcium metabolism. Rats were chosen as the experimental animals, but it is hoped that the results possess a general biological significance. In order to insure that observed differences would be the result only of a difference in utilizability of the calcium in the rations and not the result of a difference in the amount of food or of calcium consumed, the paired-feeding method was used. Eight pairs of rats were fed for 30 days on rations differing only in the fact that most of the calcium was derived in one case from raw milk and in the other case from pasteurized milk. In order to demonstrate possible small differences in the utilization of the calcium in these food products, the daily calcium intake of the rats from the milk supplement was restricted at all times to an amount no more than one-half of that normally retained during the period of rapid growth, as determined by Sherman and MacLeod (9).

The basal ration fed to all rats was designed to be poor in calcium, but to be otherwise adequate for normal growth. It contained dried whole egg to provide 18 per cent of protein, 10 per cent of sucrose, 10 per cent of lard, 7 per cent of dried yeast, 2 per cent of cod liver oil, 2.84 per cent of the Osborne and Mendel salt mixture from which the calcium salts had been removed, and enough corn starch to complete 100 per cent. This ration contained 0.144 per cent of calcium.

From the analyses of Sherman and MacLeod it may be computed that, in the weight interval from 45 to 200 grams, male rats put on gains containing somewhat less than 10 mgm. of calcium per gram, and female rats put on gains containing somewhat more than this amount. Hence, each pair of rats was provided daily with a milk supplement estimated to furnish from 5 to 7.5 mgm. of calcium per gram of gain made. The rate of gain of the rats was followed by daily weighings. Complete consumption of the milk supplements was insured by the withholding of water from the experimental animals.

The milk supplement of one rat in each pair consisted of raw milk and that of the other rat consisted of the same milk which had been subjected to pasteurization, and then diluted with distilled water to its original volume.

The pasteurization was effected in a glass container, in which was placed a motor-driven wooden paddle to keep the milk in continuous agitation. The glass container was immersed in a water bath, and the temperature of the milk was raised to 142°F. during a period of 20 minutes. It was maintained at this temperature for a period of 30 minutes, after which it was diluted with water to its original weight and thoroughly shaken before removal from the pasteurizing vessel in order to prevent any loss of calcium that might have been precipitated during the process.

TABLE 1

The body weights, gains and food consumptions of the paired rats

RAT NUMBER AND SEX	MILK SUPPLEMENT	INITIAL WEIGHT	FINAL WEIGHT	TOTAL GAIN	BASAL RATION CONSUMED	MILK CONSUMED
		grams	*grams*	*grams*	*grams*	*cc.*
1 ♂	Raw	49	212	163	252	387
2 ♂	Pasteurized	51	210	159	252	387
3 ♂	Raw	52	212	160	253	380
4 ♂	Pasteurized	51	211	160	253	380
5 ♂	Raw	45	206	161	270	395
6 ♂	Pasteurized	46	205	159	270	395
7 ♂	Raw	41	168	127	231	385
8 ♂	Pasteurized	40	168	128	231	385
9 ♂	Raw	39	186	147	245	356
10 ♂	Pasteurized	40	182	142	245	356
11 ♂	Raw	37	180	143	232	315
12 ♂	Pasteurized	37	169	132	232	315
13 ♂	Raw	41	187	146	252	354
14 ♂	Pasteurized	39	186	147	252	354
15 ♂	Raw	41	195	154	254	361
16 ♂	Pasteurized	43	190	147	254	361

The rats were fed individually in wire cages with screen bottoms having a mesh of approximately one-half inch. The initial and final weights of the rats were each the average of weighings taken on three consecutive days. At the end of the 30 day feeding period, the rats were killed with ether, the intestinal tracts discarded, and the remainder of the carcass submitted to chemical analysis. All samples were analyzed for moisture, fat (ether extract), ash, and calcium, the latter by a modified McCrudden method.

RESULTS OF THE EXPERIMENT. The weights and gains of the rats and the amounts of basal ration and of milk consumed are recorded in table 1. The differences in gains between paired rats are in all cases small; in five pairs, the rat receiving raw milk gained the faster. Subjecting the differences in gain between pair mates to "Student's" (10) method of statistical analysis, gives a mean difference of 3.37 grams in favor of the rat receiving raw milk, a standard deviation of differences of 3.97 grams, and a probability of only 0.030 that chance factors only produced these relations between

TABLE 2

The chemical composition of the entire carcasses of the paired rats

RAT NUMBER	MILK SUPPLEMENT	WATER	FAT	ASH	CALCIUM	CALCIUM IN ASH
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	Raw	64.2	12.6	2.49	0.621	24.9
2	Pasteurized	63.2	13.3	2.40	0.597	24.8
3	Raw	63.2	14.5	2.38	0.580	24.4
4	Pasteurized	62.3	15.3	2.47	0.558	22.6
5	Raw	56.7	21.6	2.50	0.620	24.8
6	Pasteurized	60.9	17.9	2.48	0.586	23.6
7	Raw	59.6	18.2	2.70	0.710	26.3
8	Pasteurized	60.5	18.5	2.64	0.638	24.1
9	Raw	59.0	19.4	2.43	0.639	26.3
10	Pasteurized	59.6	18.5	2.52	0.627	24.9
11	Raw	64.6	12.9	2.47	0.627	25.4
12	Pasteurized	62.8	14.7	2.63	0.646	24.6
13	Raw	58.3	18.8	2.56	0.655	25.6
14	Pasteurized	58.1	20.5	2.53	0.632	24.9
15	Raw	61.9	16.1	2.56	0.627	24.5
16	Pasteurized	59.3	15.9	2.63	0.631	24.0

paired rats. This probability is so small, representing odds of only 1 in 33, that it may be neglected. It appears justifiable to conclude, therefore, that the one difference in treatment accorded the two rats of each pair, namely, the giving of raw milk to one and pasteurized milk to the other, was instrumental in producing faster gains for the former rat. The gains of all rats were excellent, ranging from 4.4 to 5.4 grams daily.

The water, fat, ash, and calcium contents of the rats are summarized in table 2. With respect to water, fat, and ash percentages, there were no consistent differences between pair mates, or any clear indication that the

difference in the kind of milk supplement consumed affected the composition of the carcass. With respect to the percentage of calcium, the rat receiving raw milk had a higher percentage than the rat receiving pasteurized milk in 6 of the 8 pairs. Subjecting these differences to statistical analysis, gives a mean difference of 0.020 per cent in favor of the rats in all pairs receiving raw milk, a standard deviation of differences of 0.025 per cent, and a probability of 0.034 that chance factors alone operated. This probability is about on the borderline of significance. However, when the calcium content is computed on the basis of the total ash rather than on the live weight, the rat receiving raw milk gives the higher percentage of calcium in all pairs. In this case the mean difference becomes 1.09 per cent, the standard deviation 0.65 per cent, and the probability that fortuitous factors only were concerned in these comparisons is 0.0016. It may be concluded with high assurance that the pasteurized milk supported a less rapid and less complete deposition of calcium than the raw milk.

Referring again to table 2, it should be noted that the percentages of calcium in the carcasses of these rats, averaging 0.635 for the rats on raw milk and 0.614 for the rats on pasteurized milk, are considerably lower than the normal percentage reported by Sherman and MacLeod (9) for rats of approximately the same weight. These investigators report an average of 0.93 per cent calcium for a group of male rats weighing from 156 to 258 grams, and an average of 1.09 per cent calcium for a group of female rats weighing from 128 to 193 grams. The subnormal calcium contents of the rats in this experiment are conclusive evidence that their calcium intakes had been restricted much below their capacities to utilize calcium. Hence, the conditions were favorable for the demonstration of differences in the utilizability of the calcium of different rations.

The calcium metabolism data are assembled in table 3. The total contents of calcium in the bodies of the rats, presented in the third column, show that in all pairs the rat receiving raw milk contained the greater absolute amount of calcium, averaging 53.75 mgm. more. The standard deviation of the differences between pair mates is 23.25 mgm. and the probability that chance alone accounted for this series of paired differences is only 0.0002. The calcium retention of the rats during the 30 days of experimental feeding (column 7) was estimated on the assumption that, at the start of the experiment, the male rats contained 0.69 per cent of calcium and the female rats 0.74 per cent (9). These retention figures also give a consistent advantage to the rat in each pair that received raw milk, averaging 55.5 mgm. The standard deviation of the eight differences between pair mates is 24.8 mgm. and the probability that chance factors produced these differences is only 0.0003, surely small enough to neglect.

The total calcium intakes of the rats are also given in table 3. The milk furnished 56 to 60 per cent of the calcium consumed, averaging 57 per

cent. The calcium intake was really lower than anticipated, averaging 5.7 mgm. per gram of gain, due in part to the fact that the milk used was lower in calcium (averaging 0.132 per cent) than the value used (0.160 per cent) in controlling the intake of milk.

The total estimated retentions of calcium have been related to the calcium intakes in the last column of table 3. The surprisingly high estimates for the percentage of the calcium intake that was retained in the

TABLE 3

The calcium metabolism of the rats

RAT NUMBER	MILK SUPPLEMENT	CALCIUM CONTENT	CALCIUM INTAKE			ESTIMATED CALCIUM RETENTION	PERCENTAGE OF INTAKE RETAINED
			Basal ration	Milk	Total		
		grams	*gram*	*gram*	*gram*	*gram*	*per cent*
1	Raw	1.224	0.363	0.511	0.874	0.886	101
2	Pasteurized	1.170	0.363	0.511	0.874	0.818	94
3	Raw	1.137	0.364	0.502	0.866	0.778	90
4	Pasteurized	1.094	0.364	0.502	0.866	0.742	86
5	Raw	1.171	0.389	0.521	0.910	0.861	95
6	Pasteurized	1.095	0.389	0.521	0.910	0.778	85
7	Raw	1.072	0.333	0.509	0.842	0.769	91
8	Pasteurized	0.970	0.333	0.509	0.842	0.674	80
9	Raw	1.111	0.353	0.469	0.822	0.842	102
10	Pasteurized	1.060	0.353	0.469	0.822	0.784	95
11	Raw	1.035	0.334	0.414	0.748	0.780	104
12	Pasteurized	1.014	0.334	0.414	0.748	0.759	101
13	Raw	1.120	0.363	0.466	0.829	0.837	101
14	Pasteurized	1.080	0.363	0.466	0.829	0.811	98
15	Raw	1.128	0.366	0.476	0.842	0.845	100
16	Pasteurized	1.085	0.366	0.476	0.842	0.788	94

body are noteworthy. The average percentages are 98 for the rats on raw milk and 92 for the rats on pasteurized milk. In attempting to obtain some confirmation for this high indicated ability of the animal organism to utilize dietary calcium, a search was made of the literature for experimental data of similar import, bearing in mind that such high percentage retentions can be expected only where the intake of calcium is severely restricted. Sherman and Hawley (11) fed varying amounts of calcium, mainly in the form of milk, to three children, but even at the lowest levels of intake, the

percentage calcium retention did not exceed 73 for the oldest child, and 32 and 46 for the others. The experiments of Bloom (12), undertaken to determine the effect of crude fiber on the retention of calcium and phosphorus by growing rats, afford a much more complete confirmation of the results just reported. In experiment B, on diets containing about 0.4 per cent of calcium derived almost entirely from dried whole milk, retentions of 88 and 93 were obtained in one-week balance periods on two groups of three rats each. Similarly high percentage retentions of calcium must have occurred in the interesting experiments of Tso (13), who succeeded in raising rats normally and securing four generations on a ration containing only 0.15 per cent of calcium. Tso attributed the success of his experiments to the inclusion in his diet of 5 per cent of fresh egg yolk. It is possibly significant that the rations used in the authors' experiments contained considerable amounts of egg yolk.

In unpublished experiments[1] from this laboratory, rats were kept for a 40 day feeding period on a ration containing only 0.017 per cent of calcium, some receiving added calcium supplements and some receiving none. Five control rats, litter mates of the experimental rats, were killed at the beginning of the experiment and analyzed for calcium. At the end of the feeding period the experimental rats were killed and analyzed also, after gaining from 63 to 78 per cent of their initial weights. From these data, together with the measured intakes of food, it was possible to compute the retention of calcium as a per cent of the intake. The 4 rats that received no calcium supplement and only 1.7 mgm. of calcium daily in the basal ration, gained an average of 1.5 gram daily; on slaughter they were observed to have bones so deficient in calcium as to be very pliable and to have the appearance of cartilage. Their bodies contained an average of 0.55 per cent of calcium for body weights averaging 160 grams. These rats apparently retained an average of 96 per cent of the calcium consumed. Another group of 4 rats that received daily 21 mgm. of calcium, mainly as steamed bone meal, retained 82 per cent, and a third group of 4 rats, receiving a calcium carbonate supplement supplying an average of 22 mgm. of calcium daily, retained 78 per cent in their bodies.

Discussion. It seems difficult to account for the lowered calcium utilization on the pasteurized milk diet as compared with the raw milk diet on the basis of available information. It is true that a number of investigators have shown that pasteurization decreases the diffusibility of milk calcium (14), (15) and decreases its solubility (16), possibly due, as Magee and Harvey suggest, to the formation of colloidal $Ca_3(PO_4)_2$ from the soluble $CaHPO_4$, but there is no urgent reason to believe that this change would

[1] This work was done in 1925 by C. I. Bray, at that time a graduate student in the Division of Animal Nutrition.

appreciably affect the value of the calcium for growth. In unpublished experiments from this laboratory we have not been able to detect, in balance experiments on growing pigs, any differences in biological availability between the dicalcium phosphate and the tricalcium phosphate.

Another possible explanation is that the heat of pasteurization has destroyed or partially destroyed some unknown constituent of milk, responsible for the high availability of its calcium. The vitamin C of milk is thus affected by pasteurization, but the prevailing opinion seems to be that rats do not need vitamin C for normal nutrition. It is true that there is no unanimity of opinion on this point (17), (18), (19), (20), but certainly no clear-cut demonstration of the indispensability of vitamin C for the nutrition of the rat has yet been produced. This is merely negative evidence, it may be pointed out, and does not preclude the possibility that vitamin C is actually needed for some physiological function in the rat. In guinea pigs it seems to be concerned in the proper nutrition of the bones (22), (23), (24). The basal diet in our experiment was probably extremely deficient in vitamin C, since egg yolk has not been shown to contain it (21). For the present, therefore, it can be concluded only that the calcium of pasteurized milk is somewhat less available to the rat for growth than the calcium of raw milk. The size of this difference may be obtained from the estimates of calcium retention given in table 3. The rats on pasteurized milk stored an average of 93 per cent as much calcium as the rats on raw milk, the individual percentages for the eight pairs of rats being 92, 95, 90, 88, 93, 97, 97, and 93.

The fact that the growing rat on a restricted intake of calcium can retain under favorable conditions as much as 98 per cent of the calcium consumed carries interesting implications. It means not only that the processes of absorption are close to 100 per cent efficient, and that the kidney withdraws only minimal amounts of calcium from the blood under these conditions, but it also must mean that in the growing rat, and possibly in all growing animals, there is no integral requirement of calcium for maintenance. In the adult, the maintenance requirement for calcium may well represent merely a leakage of calcium ions through the kidney and the walls of the intestinal tract, due entirely to physico-chemical processes. Calcium ions liberated in the course of catabolism from functional combinations in the tissues and body fluids are probably just as available for the reconstruction of such combinations as are calcium ions picked up from the intestinal tract. In the young growing animal these "used" ions as well as the calcium ions coming directly from the alimentary tract may be absorbed and retained by the developing bones and the growing tissues at such a rapid rate that, at low levels of calcium feeding, the threshold of excretion by the kidneys and the intestines is never reached.

CONCLUSIONS

The pasteurization of milk slightly lowers the availability of its calcium for the growing rat, possibly by destroying some unknown constituent of the milk that favors its utilization in the animal body. Under the conditions of this experiment, there was a depression of 7 per cent in the utilization of the calcium of the experimental diet, 57 per cent of which was derived from the milk supplement. This would mean a reduction of 12.3 per cent in the availability of the milk calcium itself.

Under conditions of a severely restricted calcium intake, growing rats may retain as high as 98 per cent of the calcium consumed. The implications of this fact with respect to maintenance requirements of calcium are discussed.

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THE INFLUENCE OF GLUCOSE AND OF FRUCTOSE ON THE EFFECTIVE DEAD SPACE IN HUMAN RESPIRATION[1]

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The determination of the dead space and its relation to the estimation or calculation of the composition of the alveolar air have been the objects of a number of investigations. The results have been of two classes: on the one hand, constancy in volume of dead space (Krogh and Lindhard, 1913, 1917) and, on the other, variation of size according to depth of respiration (Douglas and Haldane, 1912; Haldane, 1915; Henderson, Chillingworth and Whitney, 1915). A recent statistical analysis by Enghoff (1931) led to the conclusion that the dead space varied according to the depth of respiration.

The data collected in the experiments in two recent publications from this laboratory (Carpenter and Lee, 1933) furnish the factors for the calculation of the average effective dead space in breathing at rest. The data necessary for this calculation are the total ventilation of the lungs per minute, the respiration rate per minute, the total carbon dioxide elimination per minute, and, when the dead space from the alveolar oxygen is calculated, the total oxygen consumption per minute, as well as the alveolar carbon dioxide and oxygen deficit percentages. For the calculation of dead space from the carbon dioxide the following formula is used:

$$\text{Dead Space} = \frac{\text{Total ventilation per min.} - \dfrac{\text{cc. } CO_2 \text{ per min.}}{\text{Per cent alveolar } CO_2}}{\text{Respiration rate}}$$

and for the oxygen dead space, the following is used:

$$\text{Dead Space} = \frac{\text{Total ventilation per minute} - \dfrac{\text{cc. } O_2 \text{ per min.}}{\text{Per cent alv. } O_2 \text{ deficit}}}{\text{Respiration rate}}$$

The method of obtaining the alveolar air has been described in a previous publication (Carpenter and Lee, 1933). The total ventilation was meas-

[1] A preliminary communication was presented at the annual meeting of the American Physiological Society, Montreal, April, 1931. THIS JOURNAL, 1931, xcvii, 509.

ured by passing the expired air through a wet meter and the respiration rate was recorded graphically from the movement of a spirometer in an open circuit apparatus (Carpenter and Fox, 1931). The following measurements are independent of one another: total ventilation, respiration rate, composition of expired air, and composition of alveolar air, and therefore such mathematical relationships as are found would seem to be of physiological significance.

TABLE 1

The effective personal dead space in experiments with J. C. expressed as percentage of volume per respiration (tidal air)

DATE	CALCULATED FROM PER CENT ALVEOLAR CO_2			CALCULATED FROM PER CENT ALVEOLAR O_2 DEFICIT		
	Range	Average	S.D.	Range	Average	S.D.
1930	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Post-absorptive:						
April 16	43.7–46.9	45.8	0.82			
April 18	48.1–51.4	49.4	1.00			
May 7	48.6–53.7	51.1	1.39	48.7–52.3	50.5	1.07
May 16	44.7–50.6	47.2	1.37	42.1–50.5	46.8	2.37
Average	46.3–50.7	48.4	1.15	45.4–51.4	48.7	1.72
25 grams glucose:						
April 30	44.8–50.0	46.8	1.72			
May 5	44.6–50.1	46.9	1.61	42.5–50.8	46.0	2.05
May 9	45.5–50.0	48.0	1.26	41.4–48.4	45.3	2.05
May 14	44.2–50.9	46.7	1.72	43.2–49.1	46.1	1.67
Average	44.8–50.3	47.1	1.58	42.4–49.4	45.8	1.92
25 grams fructose:						
April 21	45.4–50.6	47.2	1.50	43.3–48.9	45.9	1.79
April 23	41.6–50.0	44.8	2.21	40.7–47.7	43.6	2.07
April 28	44.3–48.4	46.3	1.23	40.5–46.5	44.6	1.53
May 12	44.8–48.1	46.3	0.99	42.3–53.2	46.3	2.37
Average	44.0–49.3	46.2	1.48	41.7–49.1	45.1	1.94

All the volumes measured and derived are calculated to 0° dry and 760 mm. The samples of alveolar air were obtained at the end of a normal expiration. They, therefore, probably do not represent the absolute average composition of the alveolar air. Our object was not so much to obtain the absolute level as to determine whether there were changes in the alveolar air as the result of the ingestion of sugars.

There was an instrumental dead space of 20 cc. which was subtracted from the values obtained by the calculations described, before calculating the percentage values of the volume per respiration as dead space.

The relation of the effective dead space to the volume per respiration. Results of calculations. The results of calculating the volumes of dead space in relation to the volume per respiration are given in table 1, in which the range, average, and the standard deviation of the per cent of the volume per respiration as dead space are given.

Dead space calculated from alveolar carbon dioxide. Post-absorptive experiments (no dose). The first series of observations were with J. C., post-absorptive, sitting in an arm chair, covering a base-line hour of four 15-minute periods, followed, after an interval of rest, by a continuous series of ten 15-minute periods. The range in the volumes of dead space in the four experiments was from 168 to 210 cc. with ranges in the volumes per respiration of 364 to 411 cc. There is not a wide variation in the percentage of the volumes per respiration as dead space. The respiration rate of this subject in the four experiments was nearly uniform in each experiment and the averages varied from 15.2 to 17.3 per minute. A reliable value for the alveolar air carbon dioxide percentage could be obtained with this subject in the post-absorptive condition, sitting, by applying a flat 48 per cent to the volume per respiration and by the use of the other factors derived in the measurements of the respiratory exchange, that is, total ventilation, respiration rate and carbon dioxide elimination.

Experiments with glucose. The second series with this subject consisted of 4 experiments with a preliminary base-line hour and ten 15-minute periods after the ingestion of 25 grams of glucose in 200 cc. of water at 37°. The range in dead space was from 179 to 208 cc. and in the volume per respiration from 390 to 449 cc. in the 44 periods of the 4 experiments. The experiments differ from the preceding group in that there was a rise in the respiratory quotient and an increase in the carbon dioxide elimination as the result of the ingestion of the glucose. The highest volumes per respiration (437 to 449 cc.) occurred in the third and fourth 15-minute periods after the ingestion, but this peak in no way affected the percentage relationship between the dead space and the volume per respiration. Therefore, with this subject a reliable alveolar carbon dioxide could be obtained by the use of 47 per cent of the volume per respiration as dead space.

Experiments with fructose. The third series with J. C. consisted of four experiments with 25 grams of fructose conducted in the same sequence of periods as in the group at rest post-absorptive and in the group with 25 grams of glucose. The results for the dead space are essentially the same as for the two preceding groups. The ranges in the dead space volumes are from 170 to 216 cc. and in the volumes per respiration from 375 to 478 cc. The respiratory quotient and the carbon dioxide elimination rose more markedly in this group than in the preceding group with glucose. These increases were also paralleled by more marked changes in the volume per respiration. The ranges of the volume per respiration from the last two

periods of the base-line hour to the maximum after the ingestion of fructose are as follows in the four experiments: April 21, 417 to 472 cc.; April 23, 386 to 445 cc.; April 28, 399 to 452; May 12, 417 to 478 cc. There were thus noticeable increases in the depth of respiration, which, however, did not affect the percentage relationship between the dead space and the volume per respiration. We may therefore conclude that we would be justified in the absence of direct determinations of the alveolar carbon dioxide, in calculating this value by the use of 46 per cent of the volume per respiration as dead space in this series.

In the three series the average per cent of the volume per respiration as dead space varied from 46.2 in the fructose series to 48.4 in the post-absorptive series. The average of all is a round number of 47 per cent. An accurate estimation of the relative variations of the carbon dioxide of the alveolar air for this subject under these conditions could be obtained by the use of this value in the three series of experiments.

The dead space calculated from the alveolar oxygen deficit. The dead space was also calculated from the alveolar percentage oxygen deficit and the ranges, averages, and standard deviations of the values of dead space calculated as per cents of the volume per respiration are shown for all of the experiments in table 1.

The ranges in the dead space values were slightly wider than for the values derived from the alveolar carbon dioxide. They are, however, of about the same order of magnitude, as the average percentage figures are not far from those obtained from the carbon dioxide. In general the variations in the dead space calculated from the oxygen deficit follow parallel to the variations in the dead space calculated from the carbon dioxide. Occasionally there are lower values in single periods with the oxygen dead space than with the carbon dioxide dead space. In general the carbon dioxide dead space is slightly higher than the oxygen dead space. In three experiments the two averages are identical and the greatest difference in any of the experiments is 3 per cent. The generally good agreement of the oxygen dead space and the carbon dioxide dead space with J. C. indicates that the alveolar air samples were properly collected and also properly analyzed, otherwise we would not be able to have the good agreement that is shown in the entire series. The calculation of dead space involves so many different factors that an error in any one of them is bound to lead to erroneous results. The weakest link is the collection of the alveolar air samples. This depends much on the skill of the operator and on the coördination of the subject. J. C. gave uniform results because his physiological functions in respiration are for the most part uniform, and in addition to that he was not in any way annoyed by the experiment. He was a trained individual who had had many years' experience as a subject for all types of respiration apparatus.

These observations indicate clearly that with some subjects the dead space varies as the volume per respiration even when the variations in the volume per respiration are not large. It is not the respiration rate *per se* which determined the dead space but rather the volume per respiration. The volume per respiration varies, other factors being equal, with the respiration rate. The volume per respiration also varies with the amount of carbon dioxide to be eliminated and the respiratory quotient, especially when sugars are ingested which result in both an increase in the elimination of carbon dioxide and in the respiratory quotient.

Correlation coefficients between tidal air and dead space. In table 2 are given the average values for the tidal air and for the dead space calculated by the two methods and the correlation coefficients between the tidal air and dead space calculated by the carbon dioxide method and by the oxygen method.

The tidal air is, on the whole, smaller in the group with no dose than in the group with 25 grams of glucose and the standard deviations are also somewhat smaller than in the two groups with the sugars. The dead space values are, in a similar manner, smaller with the no dose series than with the two series with the sugars.

The correlation coefficients between the tidal air and the dead space derived by the use of carbon dioxide are highly positive and significant in three out of the four experiments with no dose and positive in all four experiments with 25 grams of glucose, although not more than 6 times the probable error. In the four experiments with 25 grams of fructose they are all positive and significant. Thus, there is in all but one experiment a positive correlation between the tidal air and the dead space, that is, as the tidal air varies the dead space varies.

The correlation between the tidal air and the dead space when calculated by the oxygen method is positive in all the experiments for which the data are available but not highly significant. In only one experiment with 25 grams of glucose and the four experiments with 25 grams of fructose can any significance be attached to the correlation. In general, however, the signs are all in the same direction and indicate that the dead space when calculated by the use of the oxygen deficit varies in the same direction as the tidal air.

Variations of the alveolar carbon dioxide calculated with constant percentage dead space and with a constant volume of dead space from the values directly determined (experiments with fructose). In table 3 are given the differences between the values of the alveolar carbon dioxide directly determined and those calculated by the use of a constant *percentage* of the volume per respiration as dead space and the differences between the directly determined values and those calculated by the use of a constant *volume* as dead space. The experiments selected are those in which 25 grams of

TABLE 2

Correlation coefficients between tidal air (volume per respiration) and dead space of J. C.

Date	TIDAL AIR		DEAD SPACE				CORRELATION COEFFICIENTS BETWEEN TIDAL AIR AND DEAD SPACE			
			CO_2 method		O_2 method		CO_2 method		O_2 method	
	Average	S.D.	Average	S.D.	Average	S.D.				
1930	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*r*	*P.E.r.*	*r*	*P.E.r.*
No dose:										
April 16	384	5.8	176	4.4			+0.699	±0.096		
April 18	379	10.3	187	7.3			+0.871	±0.043		
May 7	395	6.1	202	4.7	199	3.7	+0.062	±0.186	+0.217	±0.178
May 16	393	7.2	185	7.4	184	9.6	+0.730	±0.084	+0.264	±0.168
25 gm. glucose:										
April 30	411	13.9	192	7.4			+0.486	±0.138		
May 5	422	13.4	198	7.3	194	7.7	+0.506	±0.134	+0.225	±0.171
May 9	416	12.1	199	5.4	188	7.0	+0.562	±0.123	+0.087	±0.179
May 14	421	11.4	196	7.7	194	8.9	+0.459	±0.142	+0.623	±0.110
25 gm. fructose:										
April 21	430	16.5	203	6.8	197	6.6	+0.629	±0.113	+0.413	±0.155
April 23	401	20.3	180	10.8	175	11.6	+0.612	±0.117	+0.699	±0.096
April 28	413	21.1	192	9.5	184	10.5	+0.859	±0.049	+0.803	±0.066
May 12	432	21.9	200	9.4	200	13.0	+0.907	±0.033	+0.658	±0.106

TABLE 3

Variations of alveolar carbon dioxide calculated with a constant dead space and with a percentage dead space from the directly determined values

J. C.

APRIL 21		APRIL 23		APRIL 28		MAY 12	
Constant dead space	Percentage dead space	Constant dead space	Percentage dead space	Constant dead space	Percentage dead space	Constant dead space	Percentage dead space
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
−0.01	+0.04	+0.88	+0.16			+0.08	+0.01
−0.17	−0.17	+0.20	−0.09	+0.24	−0.05	+0.15	+0.12
		25 grams of fructose					
−0.02	+0.04	+0.42	+0.11	+0.29	+0.07	−0.09	+0.08
−0.24	+0.05	−0.06	+0.19	−0.25	+0.18	−0.40	+0.19
−0.48	+0.14	+0.20	+0.58	−0.22	+0.21	−0.42	+0.28
−0.19	+0.11	+0.31	+0.43	−0.21	+0.11	−0.35	+0.17
−0.39	−0.05	+0.49	+0.39	−0.33	−0.11	−0.19	+0.11
−0.22	+0.04	+0.79	+0.48	+0.38	+0.31	−0.22	+0.07
−0.15	+0.18	+1.08	+0.64	+0.58	+0.35	+0.28	+0.22
		+0.75	+0.39	+0.27	−0.03	+0.46	+0.26
+0.02	+0.05	+0.85	+0.29	+0.24	+0.05		
−0.01	+0.02	+0.87	+0.28	+0.31	+0.12	−0.14	−0.05
Avg. ±0.17	±0.08	±0.58	±0.34	±0.30	±0.14	±0.25	±0.14

fructose were ingested as this group gave the greatest changes in respiratory quotient, total ventilation and volume per respiration. The average percentage found in all experiments (47 per cent) was used. To this was added 20 cc. instrumental dead space. The value of 215 cc. was used for J. C. in the calculation with constant volume.

The comparisons may be considered in several different ways. The averages of the alveolar carbon dioxide values as calculated by the two methods may first be compared. With J. C. the differences between the average alveolar carbon dioxide directly determined and those found by the use of a constant *percentage* (i.e., 47 per cent) dead space were 0.05, 0.32, 0.11, and 0.13 per cent. The differences between the average alveolar carbon dioxide directly determined and that found by the use of a constant *volume* (i.e., 215 cc.) of dead space were 0.16, 0.56, 0.12, and 0.09.

The averages of the individual differences by periods may be used as another criterion of comparison. These are given in table 3 and therefore need not be repeated. With J. C. the averages of the differences between the alveolar carbon dioxide values directly determined and those calculated by the use of a constant *percentage* are all smaller than the differences between the directly determined values and those calculated by the use of a constant *volume* of dead space.

A third method of comparison which is the most significant is the range of individual differences with respect to sign for the two methods of calculation. For example, on April 21, the range by the first method (constant percentage) is from -0.17 to $+0.18$, a total range of 0.35; by the second method (constant volume) the range is from -0.48 to $+0.02$, a total range of 0.50. The total ranges of variations from the determined values for the first method (constant percentage) are 0.35, 0.67, 0.46, and 0.33 and for the second method (constant volume) 0.50, 1.14, 0.91, and 0.84 per cent.

All three methods of comparison, particularly the second and third, show that more consistent and reliable sets of values of alveolar carbon dioxide could be obtained by the use of a constant *percentage* of the volume per respiration as the effective dead space than by the use of a constant *volume*.

The quantity of fructose ingested was such that not very large changes in the respiratory quotient and total ventilation were produced. With larger quantities of fructose calculations of the alveolar carbon dioxide by the use of a constant dead space with J. C. would be of value. The results in this paper now provide us with a method of recalculating past experiments with this subject on the influence of glucose and fructose on the alveolar carbon dioxide.

SUMMARY

The effective dead space in breathing was calculated from the alveolar air, the respiration rate, the respiratory exchange and the total ventilation

in experiments with an exceptionally well-trained subject. The experiments were of three groups, 1, post-absorptive, 2, before and after the ingestion of 25 grams of glucose, and 3, before and after ingestion of 25 grams of fructose.

The carbon dioxide dead space averaged 47 per cent of the volume per respiration and did not vary widely in the different groups of experiments.

The oxygen dead space averaged closely to the carbon dioxide dead space and showed about the same range of variations as the carbon dioxide dead space.

The use of a constant percentage of the volume per respiration as dead space for the calculation of alveolar carbon dioxide gave values which agreed more consistently with the values directly determined than those calculated by the use of a constant volume of dead space. This finding makes it feasible to calculate the alveolar carbon dioxide in previous studies with the same subject on the effect of sugars on the respiratory exchange.

We wish to acknowledge the assistance of Mr. W. H. Leslie by whom were made all the calculations.

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AN EXPERIMENTAL STUDY OF A SO CALLED "PELLAGRA-PRODUCING" DIET

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It was reported in a previous publication (1) that five pellagrins showed improvement of their skin lesions while receiving a diet calculated to be low in minerals, proteins, and vitamins C, D, and G. Since vitamins A, C, D, E, and F are not believed to bear any relationship to the development of dermatitis, our chief interest was centered on the vitamin G content of this diet. Some investigators (2) consider the lack of vitamin G (B_2), the heat stable portion of the vitamin B complex, the sole cause of pellagra. Since it is well known that a deficiency of this vitamin produces a fairly definite picture in young albino rats (3), (4), it seemed advisable to test the actual deficiency of the diet by animal experimentation. Some writers believe that the vitamin G deficiency disease in rats is an analogue of pellagra in human beings (2), (3), but other investigators do not accept this view (5), (6).

Evidence is presented in this report showing that the administration of the experimental diet alone to rats produces the characteristic picture of vitamin G deficiency (emaciation, alopecia, dermatitis, and swelling around the eyes). The addition of ventriculin supplements is shown to protect against the deficiency or to cure it when once developed.

MATERIAL AND METHODS. Twenty-four albino rats, seven weeks of age, were chosen from four litters. The rats were heterogeneously divided into four groups of six each. The total initial weight of the six rats in groups I, II, III, and IV was 525 grams, 550 grams, 535 grams, and 550 grams respectively. A mixture of corn meal, pork fat, artificially colored (synthetic) maple syrup, polished rice, cornstarch pudding, and sugar was made so that each constituent was in the same relative proportion as in the diet previously given to the pellagrins. A small amount of water was added and the entire mass thoroughly mixed. It was then slowly heated until dry and ground into small pieces by means of a food chopper.

The animals in all groups received the basal diet ad libitum but additional

daily supplements of 1.5 gram of ventriculin, yeast, and casein[1] were given to each animal in groups II, III, and IV respectively. All rats were weighed semi-weekly and the amount of food ingested was determined daily (table 1 and 2). When the six rats in group I developed the characteristic appearance of vitamin G deficiency, three of them, showing the most marked lesions, were separated and given ventriculin in daily doses of 1.5 gram each until they appeared normal.

TABLE 1

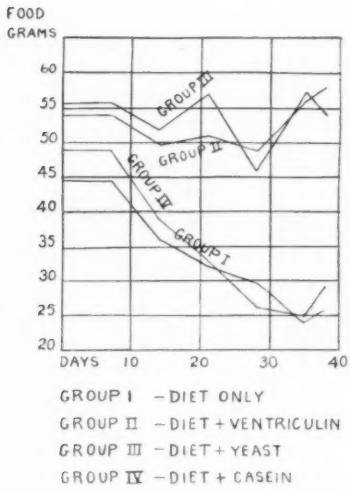

TABLE 2

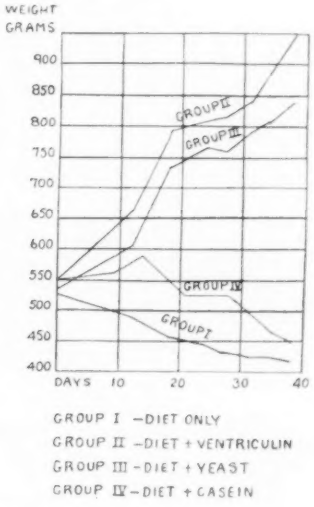

TABLE 1

Showing relative food intake of the rats receiving the diets

Note the animals that were given ventriculin and yeast ingested more food than those receiving the basal diet or the basal diet and casein.

TABLE 2

Showing relative weights of the groups of animals

Note those that received the basal diet and yeast or ventriculin continued to grow in contrast to those receiving only the basal diet or the basal diet and casein.

OBSERVATIONS. By the end of the sixth week small, symmetrical areas of alopecia developed over the head, neck, shoulders, and legs of the animals receiving the basal diet alone. During the next two weeks the head, neck,

[1] This casein was made free of vitamin G after the method of Osborne and Mendel and in addition it was further treated by boiling with large quantities of ninety-five per cent alcohol which should inactivate any of the remaining vitamin.

trunk, and limbs became denuded. At the same time the skin reddened over the ears, legs, hind paws and forepaws and the ears thickened. Following the initial loss of hair the skin became moist and later dry and encrusted. Progressive loss in weight continued throughout the experiment. Three of the animals had circumocular swelling by the end of the eighth week. Toward the end of the experiment the animals suffering from the deficiency had a low food intake (see table 1) but they remained active and showed no evidence of paralysis. They assumed a characteristic position and when left unmolested in the cages, squatted with the body bent forward, the hind legs widely separated and the fore paws under the chest, giving an exaggerated hump-back appearance.

The three severely diseased animals taken from group I rapidly improved following the administration of ventriculin. The hair quickly reappeared over the depilitated areas and their weight increased throughout the remainder of the experiment. When they seemed normal in every respect they were placed on a regular stock diet. The three test animals in group I

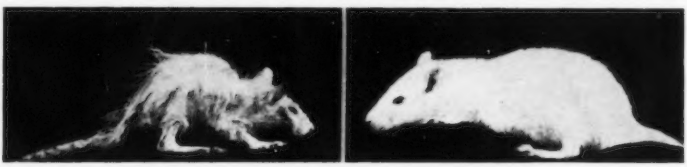

Fig. 1 Fig. 2

Fig. 1. Showing the emaciation and alopecia present in all animals of group I.

Fig. 2. Showing the same rat as photograph 1, three weeks later. Note improvement in body weight and return of hair following the administration of ventriculin.

that did not receive ventriculin died during the interval between the ninth and thirteenth week of the experiment. It is noteworthy that the rats receiving daily supplements of casein (group IV) developed changes comparable to those described in the rats eating the basal diet alone. These changes were delayed, however, similar lesions appearing three weeks later. After the manifestations of the deficiency were observed, these animals, like the ones receiving the basal diet only, became progressively worse. In contrast, the animals receiving the basal diet and additions of yeast (group III) or ventriculin (group II) remained healthy throughout the course of the study.

Discussion. It has been shown in the experiments reported here that the restricted diet administered to pellagrins with improvement of their skin lesions was inadequate for the proper nutrition of young albino rats. These animals lost weight, developed alopecia and reddening of the skin over the body, ears, and legs. These changes and the characteristic manner in which they rested in the cage gave the picture of a vitamin G deficiency

In addition, it was shown that yeast and ventriculin protected against the deficiencies in this diet whereas casein did not. The observations in this experiment that casein did delay the onset of the disease are in keeping with those of previous investigators (7). Further experimentation will probably determine that the changes observed in these animals were due to the low vitamin G content of the diet.

Sharp (8) and Sturgis and Isaacs (9) have shown that ventriculin contains a substance capable of remitting pernicious anemia. The present experiment demonstrates that ventriculin has curative properties (see photographs 1 and 2), remitting the manifestations of vitamin G deficiency or some closely related dietary disease. We realize that ventriculin is a mixture of chemical substances and therefore do not make any speculations as to the identity of the specific materials in it which are efficacious in the treatment of the dietary deficiency condition. Since ventriculin remits both diseases we feel that the antianemic factor is probably closely related to the substance protecting and curing the rats suffering from the pellagra-like disease. This interpretation is supported by the recent work of Strauss and Castle (10) who have found that the extrinsic factor in the pernicious anemia state is vitamin G or some closely related substance.

SUMMARY AND CONCLUSIONS

1. It has been shown in the experiments reported here that the restricted diet administered to pellagrins with improvement of their skin lesions is inadequate for the proper nutrition of young rats.

2. These animals develop the characteristic appearance of vitamin G deficiency when they are given the basal diet alone or the basal diet plus casein. They are protected from this condition by the administration of the basal diet plus supplements of either ventriculin or yeast.

3. Ventriculin possesses striking therapeutic properties as demonstrated by the rapid and apparently complete recovery of the animals from this deficiency following the administration of ventriculin.

4. The correlation of the results obtained in the present experiments with those showing that ventriculin is curative for pernicious anemia (8), (9) suggests that the substance in the ventriculin which protects the animals from this inadequate diet may be vitamin G or some closely related substance and that this material is possibly the extrinsic factor which Castle has discussed in the development of the deficiency state of pernicious anemia.

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NATURE AND SOURCE OF FIBERS CONTRIBUTING TO THE SAPHENOUS NERVE OF THE CAT

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In work previously published on the fiber types concerned in the performance of various bodily activities (Heinbecker, 1931 and Heinbecker, Bishop and O'Leary, 1932), we have so far failed to obtain data which positively associate the non-myelinated fibers of the dorsal root with any afferent function. Hinsey and Gasser (1930) have shown that stimulation of the dorsal root fibers that give rise to the "C" wave of peripheral nerves causes vasodilatation, and these fibers are evidently the non-myelinated ones (Heinbecker, 1929). The evidence, while not sufficient to exclude all non-myelinated fibers from the afferent group, seems sufficiently suggestive to justify a functional study of the fiber groups of a typical sensory nerve, by a procedure which does not directly utilize staining methods, but which can be readily correlated with the results of histological studies. If it can be shown that fibers in a complex nerve fall into specific functional groups that can be recognized by distinct physiological properties, the interpretation of the classification based upon histological appearance may be rendered less equivocal.

It may be stated at the outset that our results confirm those of Cajal (1906), Dogiel (1908) and Ranson (1912) in respect to the origin of non-myelinated fibers from cells in the spinal ganglia. They add new evidence for the physiological similarity of the non-myelinated fibers arising from such cells and the non-myelinated fibers of sympathetic origin which also occur in skin nerves. Such physiological comparisons depend upon an established correlation between the form of a nerve's potential of activity as recorded by the cathode ray oscillograph and the histological appearance of its fibers (Bishop and Heinbecker, 1930). In any nerve where non-myelinated fibers are present, a characteristic potential wave occurs in the electroneurogram, and the size of this "C" potential wave is proportional in mixed nerves to the relative number of non-myelinated fibers present.

MATERIALS AND METHOD. The saphenous nerve of the cat was chosen. It was suitable for our purpose because of its high content of non-myelinated fibers and because of the amount of accurate knowledge available

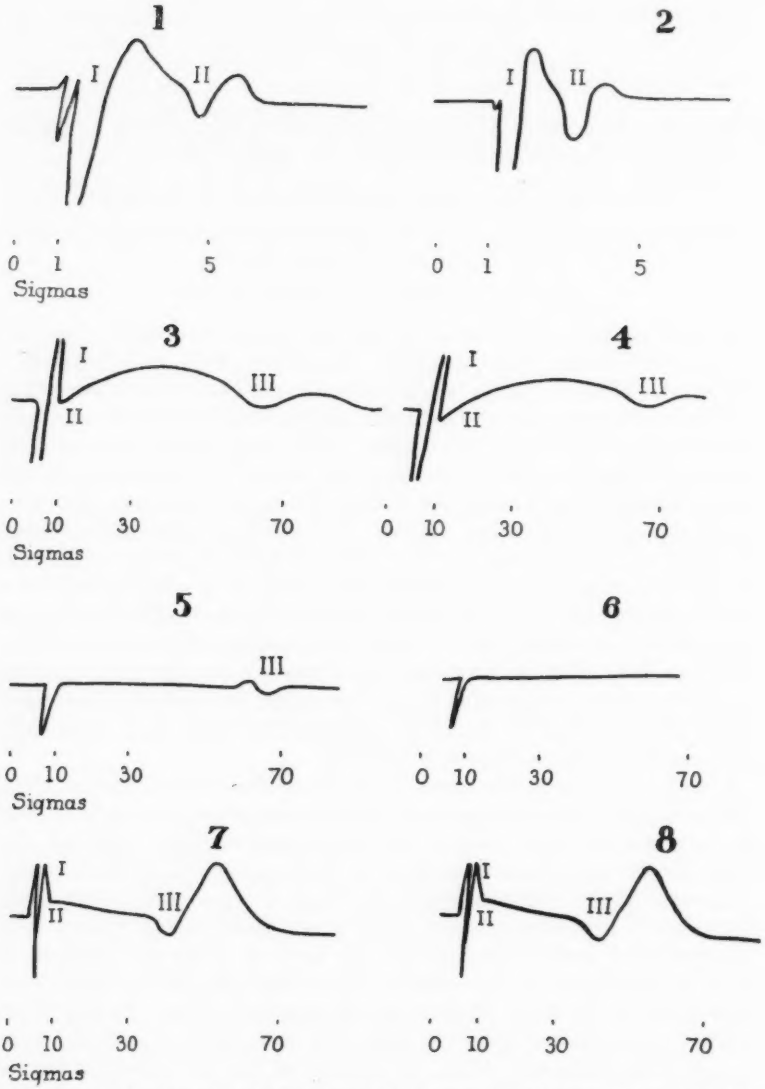

Fig. 1. 1. Conducted action potential of normal saphenous nerve (cat 51), showing the first and second potential complexes. Conduction distance 52 mm. Temperature 38°C. Amplification 100 mm. per mv. Time in sigmas in this and subsequent records.

2. Conducted action potential of saphenous nerve (cat 51) after removal of the ipsolateral sympathetic chain from diaphragm to pelvis. Conduction distance 37

concerning it. The approach to the problem was one whereby the various possible sources contributing to the nerve could be singly or conjointly eliminated. These sources are the appropriate motor and sensory roots, the spinal ganglia and the ipsolateral sympathetic chain from the diaphragm to the pelvis. In the cat the saphenous fibers are derived essentially from the fifth and sixth lumbar roots; however, roots and ganglia above and below this level were included in the operative removals.

The animals were operated upon under general anesthesia. Section of roots and removal of ganglia were accomplished after a laminectomy extending from the fourth lumbar vertebra to the sacrum. The roots were cut where the dural extension becomes inseparable from the root trunk Removal of ganglia with minimum trauma to the motor roots was evidenced by a slight residue of paralysis. The sympathetic chain was removed in one piece transperitoneally. After sufficient time for degeneration (11 to 20 days) the altered nerve trunks were studied physiologically and histologically.

The functional studies consisted of a potential analysis of the altered saphenous nerves, using as controls the normal nerves of the opposite sides. The recording mechanism was the cathode ray oscillograph and amplifier yielding a sensitivity up to 400 mm. per millivolt.

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mm. Temperature 38°C. Amplification 100 mm. per mv. A comparison of the potential from the normal nerve at the same conduction distance, amplification and deflection time with this potential showed no detectable difference.

3. Conducted action potential of normal saphenous nerve (cat 51) to show third potential component. Conduction distance 37 mm. Temperature 37.5°C. Amplification 200 mm. per mv.

4. Conducted action potential of saphenous nerve (cat 51, same nerve as used for electroneurogram 2) to show third potential component. Conduction distance 37 mm. Temperature 37.5°C. Amplification 200 mm. per mv. Note a very slight diminution in area when compared with record 3; this indicates a slight loss of fiber content of the non-myelinated type.

5. Conducted action potential of saphenous nerve (cat 98) after removal of the ipsolateral L. 4, 5, 6 and 7 spinal ganglia. A low third potential component is shown. This is derived from non-myelinated sympathetic fibers in the nerve trunk. Conduction distance 37 mm. Temperature 37°C. Amplification 200 mm. per mv.

6. Conducted action potential of saphenous nerve (cat 94) after removal of ipsolateral L. 5, 6 and 7 and S. 1 spinal ganglia, together with the sympathetic chain from the diaphragm to the pelvis. There is no action potential, the first early deflection is the result of the stimulus. Amplification 200 mm. per mv.

7. Conducted action potential, normal saphenous nerve (cat 77) to show third potential component. Conduction distance 33 mm. Temperature 37°C. Amplification 200 mm. per mv.

8. Conducted action potential of the saphenous nerve (cat 77) after section of L. 5, 6 and 7 and S. 1 and 2 motor and sensory roots centrally to the spinal ganglia. Conduction distance 33 mm. Temperature 37°C. Amplification 200 mm. per mv. Note that there is no detectable difference between potentials 7 and 8. This indicates that there is no loss of fibers in the saphenous nerve as a result of the operative procedure.

After completion of the oscillographic studies the experimental and normal saphenous nerves were divided into upper and lower segments. The upper segments were stretched slightly upon glass rods and fixed by immersion in 1 per cent osmic acid. After washing for twenty-four hours they were dehydrated and either imbedded in paraffin or double imbedded in celloidin-paraffin following the technique of Stieve (Romeis, 1928). Sections five microns in thickness were cut from the lower end. The lower segments were drawn through pieces of spinal cord of similar length (Ranson and Davenport, 1931), fixed in ammoniated alcohol and subjected to the Ranson method of silver impregnation. Cross-sections, five microns in thickness, were cut from the upper end. Thus, silver and osmic cross-sections were provided not more than 4 mm. apart.

Interpretation of functional results. The conducted electroneurogram of the cat's saphenous nerve shows three main potential maxima. The first

TABLE 1

*Thresholds, conduction rates and absolutely refractory period values associated with the three potential maxima I, II and IV of cat saphenous nerve**

POTENTIAL MAXIMUM	THRESHOLD (ARBITRARY UNITS)	CONDUCTION RATES FAST FIBERS (M.P.S.)	ABSOLUTELY REFRACTORY PERIOD (SIGMAS)
I	100	80–60	0.6 to 0.7
II	450–600	30–20	0.7 to 0.8
IV	6,000–10,000	1.0–0.5	3.5 to 6.0

* Fibers giving rise to the potential maximum III are, according to the nomenclature used by us, found typically in certain sympathetic and parasympathetic nerves (Heinbecker, 1930). They are either absent or too few in number in the cat saphenous nerve to give a recognizable potential at the amplification used (400 mm. per mv.).

of these has two minor crests corresponding to the beta and gamma waves of the sciatic (Erlanger, 1927). The gap between these and the next major elevation is only a relative one, and sometimes shows a third minor wave. The second potential is generally single and the third is single or double (fig. 1). The thresholds, conduction rates and absolutely refractory period values for the most irritable fibers of the three primary groups are given in table 1. It is to be noted that the first two potentials have properties which definitely indicate their derivation to be from somatic fibers, the third has properties similar to those of autonomic fibers. On the basis of previous correlations (Heinbecker, 1930; Bishop and Heinbecker, 1930) the fiber types responsible for the three potential maxima are known. The first is derived from the large and medium-sized, thickly myelinated fibers, the second from small and somewhat more thinly myelinated fibers and the third from non-myelinated fibers. Studies of osmicated cross-sections of normal cat saphenous nerves fail to reveal the presence of any appre-

ciable number of the small, thinly myelinated motor fibers, typically found in autonomic nerves like the cervical sympathetic and cervical vagus trunks and which are responsible for a definite potential in those nerves (Heinbecker, 1930). This potential is not recognizable in the saphenous records. In the interpretation of the potential records from experimental saphenous nerves the above criteria have been used, allowing deviations from the normal potential to be directly interpreted in terms of alterations in the fiber content of the nerve trunk.

RESULTS: *1. Motor fibers of L. 4, 5, 6, 7 eliminated.* When the L. 4, 5, 6, 7 left sensory and motor roots were ligated and cut proximal to the position of the spinal ganglia (3 cats) and an adequate time allowed for physiological and anatomical manifestations of degeneration (over twenty days in each case), no perceptible change from the normal of the potential could be detected in the experimental nerves. Osmic acid and silver pyridine preparations of each set of experimental and normal nerves were compared. No significant difference in the content of either myelinated or non-myelinated fibers was revealed (compare fig. 2, 3a and 3b with 1a and 1b). We conclude from this experiment that the number of axons which leave the gray matter of the cord to join the saphenous nerve via sensory or motor roots are too few to be recognizable by the methods employed.

2. Ablation of the dorsal root ganglia without injury to motor roots. In six cats the left L. 4, 5, 6 and 7 spinal ganglia were removed. In five of these no damage was done to the motor roots. In one, the left L. 4 motor root was completely cut across. One cat was used seven days post-operatively, the remainder were allowed to live for fifteen to twenty-three days. After careful necropsy, injury to the sympathetic rami communicating with the nerve trunks seemed excluded. In each case the potential record for the experimental saphenous nerve showed a marked change. The only portion of the nerve's potential to remain was a fraction (10 to 20 per cent) of the potential component which is normally derived from the non-myelinated fibers. The conduction rate of this potential varies from 1.0 to 0.5 meter per second under our experimental conditions. Its absolutely refractory period time falls between 3.5 and 5 sigmas and its threshold is of an order characteristically found for non-myelinated fibers.

Histological studies showed marked degenerative changes in the experimental nerves. A few intact myelinated fibers (size ranges 8–10μ and 3–5μ) were constantly observed. These were apparently too few to give a recognizable potential within the limits of sensitivity of the apparatus employed. They may have been derived from the motor roots, the sympathetic chain or dorsal roots above or below those included in the operative procedure. Intact non-myelinated fibers in significant numbers could be detected in cross- and longitudinal sections of the silver pyridine preparations. These occurred singly or in groups of two to five (fig. 3). How-

ever, the reduction in numbers (80 to 90 per cent) as compared with the non-myelinated content of the normal saphenous (compare fig. 2, 5b with 1b) was sufficient to account for the considerable reduction in the potential of the non-myelinated fibers.

3. Motor and sensory roots destroyed. Three cats were utilized to eliminate the possibility that the non-myelinated fibers which remain in the saphenous nerve after ablation of the dorsal root ganglia were derived in part or wholly from the motor roots. Motor and sensory components of the left L. 4, 5, 6, 7 nerves were crushed just distal to the spinal ganglia. The reduction of potential duplicated that obtained when the spinal ganglia alone were removed. Histological preparations proved that all of the myelinated fibers had been eliminated (fig. 2, 4a). There was a slightly more marked reduction in the number of non-myelinated fibers; but this could have been due to injury of the gray rami as well as to removal of motor root non-myelinated fibers. However, necropsy showed that the communicating rami were macroscopically intact.

4. *Removal of the ipsolateral sympathetic trunk.* Examination of the saphenous nerve from those animals in which the ipsolateral sympathetic trunk had been removed from pelvis to diaphragm (5 cats) showed a very slight reduction in potential as compared with the normal. The potential component derived from the non-myelinated fibers was lower than normal by ten to twenty per cent. There was no appreciable change in the potential normally derived from the somatic fibers. These results afford an interesting correlation with those obtained after removal of the spinal ganglia. The non-myelinated potential remaining in these sympathectomized nerves is undoubtedly derived from cells located in the dorsal root ganglia; the potential remaining after removal of the dorsal root ganglia must have its

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Fig. 2. Photomicrographs: cross-sections of saphenous nerves illustrating what remains when the sympathetic, motor and sensory components are singly or conjointly eliminated. In 1b and 2b the non-myelinated fibers appear black; the large myelinated fibers, pale. In 3b this difference is not so apparent.

1a. Normal saphenous nerve (cat 43) osmic.

1b. Normal saphenous nerve (cat 43) silver.

2a and 2b. Sympathectomized saphenous nerve of cat 43. Note that a reduction in the non-myelinated fibers is not apparent from the photomicrograph 2b.

3a and 3b. Possible motor, root fibers have been eliminated by section of the dorsal and ventral roots between the spinal ganglia and the cord (cat 77, see fig. 1, 7 and 8). As in 2a there is no apparent change in the myelinated fiber composition of the nerve. The non-myelinated fibers do not appear to be reduced in number.

4a and 4b. Sensory and possible motor root components have been removed by crushing and cutting the roots from L. 4 through L. 7 just distal to the spinal ganglia. Only sympathetic non-myelinated fibers remain.

5a and 5b. The dorsal root ganglia have been removed from L. 4–L. 7 without injury to the motor roots. A few myelinated fibers and the sympathetic non-myelinated component remain. Zeiss 8 mm., Homal 1. 425 diameters.

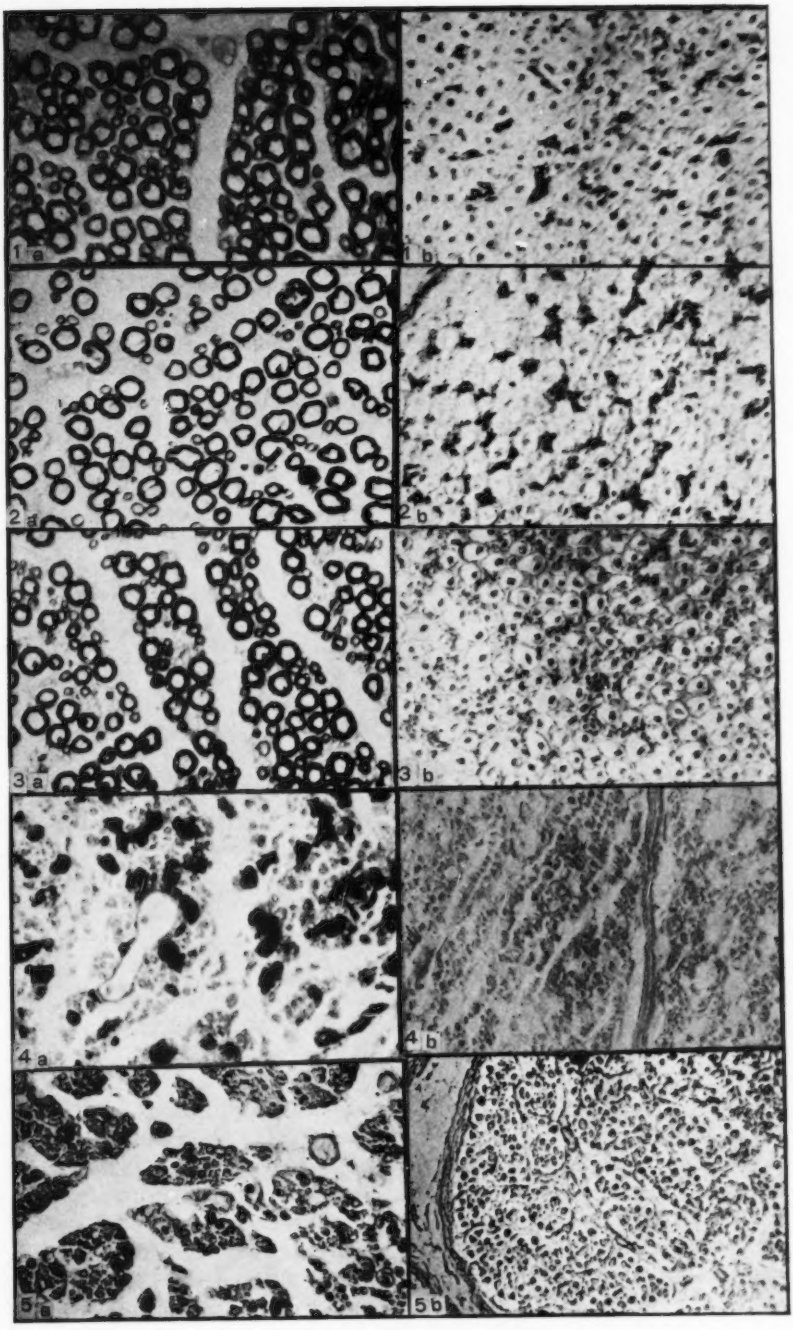

Fig. 2

origin in non-myelinated sympathetic fibers. The results indicate that the former make up from eighty to ninety per cent of the non-myelinated fibers in the nerve, the latter ten to twenty per cent. Our histological preparations do not indicate a difference in the fiber content of normal and sympathectomized nerves (compare fig. 2, 2a and 2b with 1a and 1b).

5. Removal of L. 4, 5, 6, 7 spinal ganglia and the ipsolateral sympathetic trunk. This experiment was conducted in three cats to determine whether or not intact non-myelinated fibers could still be detected in the saphenous nerve by functional or histological methods. Functionally, the potential

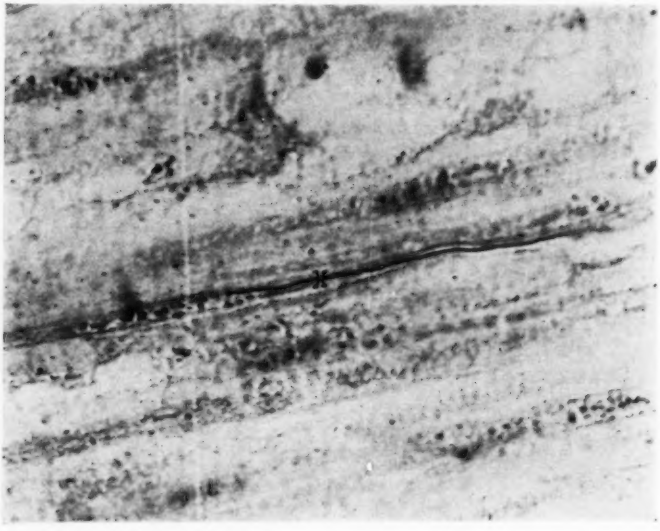

Fig. 3. Photomicrograph of a longitudinal section of a saphenous nerve sixteen days after removal of the ipsolateral L. 4, 5, 6 and 7 dorsal root ganglia. *X* indicates a sympathetic non-myelinated fiber. The dorsal root myelinated and non-myelinated fibers had undergone degeneration. Zeiss 2 mm., 1.3. Homal IV.

of activity from the experimental nerves was not sufficient to be detected. Histologically, only a few myelinated fibers remained intact.

Discussion. An analysis of our results from the functional viewpoint indicates that with the exception of a small fraction of the area of the "C" potential, the conducted action potential of the saphenous nerve entirely disappears when the L. (4), 5, 6, (7) dorsal root ganglia are ablated. The histological findings check with the functional, both as regards myelinated and non-myelinated fibers except for the presence of a few myelinated fibers of large, small and medium-size (in order of frequency) which remain

structurally intact at twenty days. The numbers of these are not considered sufficient, even if functionally as well as structurally intact, to yield recognizable potentials at the amplification employed. The origin of these fibers is open to discussion. They disappeared (in three animals) when the motor roots as well as the dorsal root ganglia were destroyed. This does not exclude their sensory origin since Windle (1931) showed that typical sensory ganglia cells may be present in motor roots. However, other explanations of the origin of certain of them are also available: they may arise from dorsal root ganglion cells interspersed among the nerve fibers peripheral to the ganglia, or they may have cells of origin in ganglia above or below the level of those ablated.

Ken Kuré and associates (1930) have reported the persistence of a majority of the myelinated fibers of size 3μ or less in the central stumps of dorsal roots transected between the ganglia and the cord. Their experiments were conducted upon dogs which were killed at intervals of thirteen days to one year post-operatively. Representative roots from each division of the spinal cord were used. They concluded that the cells of origin of these fibers occurred in each spinal segment between the ventral horn and the *substantia gelatinosa*. The group was designated as *spinal parasympathetic;* and they assigned to it trophic, vasodilator and secretory functions (to the sweat glands).

Since the evidence developed in this communication could not support the occurrence of such fibers in the saphenous nerve, we repeated their procedures upon the L. 5, 6, 7 and S. 1, 2 roots of the cat. In addition, preparations of the dorsal root entry zone were made in eight cats which had had their dorsal root ganglia ablated seven to twenty-eight days previously. The former material was prepared by the osmic acid, Weigert and pyridine silver methods; the latter by the pyridine silver alone. Our preparations (O'Leary, Heinbecker and Bishop, in press) do not give evidence that either myelinated or non-myelinated fibers persist in the central stumps of the divided dorsal roots. However, small fibers of the dorsal roots have been shown to persist apparently intact at seven days and a few regenerated fibers are apparent in animals with divided dorsal roots as early as twenty-five days post-operatively. At 100 days post-operatively (division of the dorsal roots) many newly regenerated fibers are to be observed in the dorsal roots. These are either non-myelinated or thinly myelinated.

As indicated previously, the histological supports the functional evidence, which leads us to assign to the dorsal root non-myelinated fibers the same physiological characteristics as those possessed by the non-myelinated fibers of sympathetic origin (Heinbecker, 1930). A fraction of the "C" potential (10 to 20 per cent) is elicited from the saphenous nerve when all fibers except those of sympathetic origin have degenerated (fig. 2, 4a and

4b). Eighty to ninety per cent of the "C" potential is elicited from the saphenous nerve in completely sympathectomized cats (origin: dorsal root, non-myelinated fibers). The reduction from the maximum ("C") potential when the dorsal root ganglia are ablated or the ganglia and the motor roots destroyed, coincides with the reduction in number of non-myelinated fibers which may be detected by the pyridine silver method (80–90 per cent). However, when the cat is sympathectomized and the dorsal root non-myelinated fibers remain intact, the reduction in numbers is only noticeable functionally. Histologically no difference is observed between normal and sympathectomized nerves, thus confirming the statement of Ranson and Davenport (1931). Such a difference might be demonstrated by quantitative methods. However, it is questionable whether even the most accurate possible counts of non-myelinated fibers (silver pyridine preparations) would reveal a difference beyond the allowance necessary for experimental error. Ranson and Davenport, for example, conducted several such counts of non-myelinated fibers upon a single well-fixed filament of a dorsal root. There was a difference of 20 per cent between the high and low figures, and while the counts of non-myelinated fibers in the saphenous nerve are admittedly conducted with more accuracy than in dorsal roots, this experimental error may not be neglected, particularly since the maximum reduction in the "C" component of potential in sympathectomized as compared with normal nerves is 20 per cent.

Nevin (1930) examined the skin and muscle nerves of the posterior extremities of dogs which had been subjected to (1) unilateral lumber sympathectomy and (2) ablation of the L. 5, 6, 7 and S. 1 dorsal root ganglia. The animals were kept alive for varying lengths of time post-operatively. He reached the conclusion that the small, myelinated fibers of skin nerves were of dorsal root origin and that the non-myelinated fibers had a mixed origin from the lumbar sympathetic chain and the dorsal roots. The sympathetic non-myelinated fibers were found to be more numerous than were the dorsal root fibers in both skin and muscle nerves. The dorsal root non-myelinated fibers were distributed mainly to the skin nerves where they accompanied the small, myelinated fibers. Nevin's results seem comparable with ours except insofar as the number of non-myelinated fibers contributed to the saphenous nerve by the sympathetic chain and by the dorsal roots is concerned. If the sympathetic non-myelinated fibers are the more numerous in the dog, the peripheral organization in the dog and cat must differ markedly. This we are inclined to doubt.

In nerves from which the dorsal and ventral root components had been eliminated by degeneration we were unable to detect any potential except that assignable to non-myelinated fibers. Histological preparations of

these nerves showed no myelinated fibers remaining. We thus have a further and striking correlation between the "C" potential and non-myelinated fibers of the sympathetic. Moreover, the absence of the saphenous "B" potential, believed by Erlanger and Gasser (1930) in the dog and cat to enter the femoral nerve via the sympathetic rami, proves that in the cat saphenous, at least, this potential component cannot possibly be assignable to postganglionic fibers, as they concluded, especially since this "B" potential is of fully normal size in sympathectomized nerves with intact dorsal root ganglia. This circumstance supports our previous finding that the fibers responsible for this potential are afferent (Heinbecker, Bishop and O'Leary, in press). Such preparations also demonstrate that no myelinated visceral afferents enter the saphenous of the cat from distant dorsal roots. Further, it is easily demonstrated that no pain fibers of any kind pass via the sympathetic to roots beyond the level of those sectioned in these experiments. It is well known that pinching a nerve is an adequate stimulus for pain, and in lightly anesthetized cats the slightest handling of the dorsal roots, or slight pinching of the nerve, causes violent motor responses. After cutting of the dorsal roots (L. 5–S. 2) we were unable to obtain any indications of sensory or reflex effect whatever by repeated pinching of the nerve, thus indicating that the regional dorsal roots serve as the only source of afferents in the saphenous nerve of the cat.

Our findings throw some additional light upon the question of the nature of the dorsal root fibers capable of causing vaso-dilatation. It has been shown by Hinsey and Gasser (1930) that the "C" component of the compound conducted action potential is derived in part at least from fibers responsible for vaso-dilatation. Non-myelinated fibers in sympathetic nerve trunks with similar physiological properties have been shown by Bishop and Heinbecker (1931) to be responsible for vaso-constriction. Consequently vaso-dilator and vaso-constrictor fibers can be said to belong to the same histological and physiological types.

As to the function served in the normal activity of the body by the dorsal root non-myelinated fibers, aside from the vaso-dilatation which has been inferred since Bayliss' (1901) work to be antidromic, it has been difficult to obtain a direct test by the methods we have employed for other elements, because a strength of stimulus which activates these fibers is more than sufficient to stimulate all the other fibers in a nerve, and the result is therefore confused by the multiplicity of effects. We think that the present work leads to the inference that these fibers are not sensory at all for the following reason: All the sensory fibers to which we have been able to assign a function have what we have referred to as somatic properties, and these include the fine myelinated fibers of the dorsal roots responsible for sensations of pricking pain, and for reflex rise of blood pressure and struggling reflexes. All the autonomic motor fibers, myelinated as well as non-

myelinated, that we have studied, have a quite different set of properties. There are thus two distinct types of myelinated axons, not simply distinguishable by ordinary histological tests, but quite different by physiological criteria; and only one type of non-myelinated axon. If, as Ranson (1931) suggests, the dorsal root non-myelinated fibers are to be included in a functional group with the fine myelinated afferents, one might expect them to have similar physiological properties. If, in other words, such fibers differ only in the matter of myelination, with transitional stages of non-myelinated fibers on the way to become myelinated, in the first place we should find evidence in their action potentials of *transitional properties*, especially of conduction rates, between those of non-myelinated and of myelinated axons, whereas the gap in the action potential series between these two groups in the dorsal roots and saphenous nerve is the blankest space we have found in any nerve; and secondly, we might expect that the properties of afferent non-myelinated areas, if such there be, would resemble those of *afferent* myelinated rather than those of *efferent* autonomic myelinated of a similar size. At any rate, the fact that only the non-myelinated fibers cause vaso-dilatation, whether this effect is considered to be antidromic or otherwise, points to a difference of function between this group of fibers and the myelinated group; and since pricking pain, pressor reflexes and struggling reflex movements can be specifically assigned to the myelinated group, these effects can presumably be as specifically excluded from the non-myelinated group; and the functional definition of the latter may thus be further approximated by a process of elimination.

SUMMARY

The saphenous nerve fibers of the cat are essentially of three types, large thickly myelinated, smaller and more thinly myelinated and non-myelinated. The myelinated fibers have somatic properties and all have their cells of origin in the dorsal root ganglia.

The non-myelinated fibers are derived from two sources, 80 to 90 per cent from dorsal root ganglion cells, the remainder from cells in the sympathetic chain.

The properties of the non-myelinated fibers of dorsal root origin are in every way similar to the properties of the non-myelinated fibers of sympathetic origin and strikingly distinct, therefore, from the properties of the somatic myelinated fibers.

No evidence of synapsis in the dorsal root ganglia has been obtained.

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MECHANISMS FOR DISSIPATING HEAT IN MAN AND DOG

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It may be assumed for man and the domestic animals that about one-fifth of the energy input is converted to mechanical energy, the remainder appearing as heat.[1] The dissipation of this heat depends on mechanisms which are adequate for ordinary conditions but which may fail under stress. Manifestations of this breakdown have been described by the authors both for man (2) and for the dog (3). The latter study revealed that, in respect to capacity for dissipating heat, at low temperature the dog is superior to man. There remained uncertainty whether this superiority extends to high temperatures. This uncertainty has been cleared up by recent experiments which enable us to compare the adequacy of the heat-dissipative mechanisms of man and dog at moderate and at high external temperatures.

The experiments were begun at Boulder City, Nevada,[2] during the past summer and concluded after our return to Boston. On the days selected for experiments at Boulder City the sky was clear and the shade temperature about 40°C., 20° higher than during our Boston experiment. The exercise consisted of walking, the dog being on leash. In the final Boulder City experiment, to be described in detail, the subjects walked 3.2 km. down a 5 per cent grade and returned over the same route, the round-trip requiring about 70 minutes. The dog made 4 trips covering 26 km. and the man 5, covering 32 km. (20 miles). At the end of each round-trip, blood samples were obtained. In addition to their analysis, observations were made of rectal temperature, water intake and body weight.[3] In the

[1] Reference may be made to the investigations of Benedict and Cathcart (1) for information on this subject.

[2] We are indebted to Secretary Wilbur of the United States Department of the Interior for the opportunity of carrying on a number of investigations at Boulder City during the past summer. The officers of the Bureau of Reclamation, in particular, Commissioner Elwood Mead and Mr. Walker Young, and the officers of six companies, General Contractors, facilitated our work in many ways.

[3] The figures for body weight and the derived figures for water loss are less accurate for the dog than for man. The scales used at Boulder City had an error of ¼ lb. and since the dog was weighed by difference there was a possible error of ½ lb. or 1.5 per cent of his weight. An indeterminate amount of fluid was lost in the saliva dripping from his mouth and the volume of fluid in the bladder and stomach were variable.

Boston experiment the work output was made essentially the same by suitable adjustment of the rate and inclination of a motor-driven treadmill.

The energy output for D. B. D. during the 24-hour period including the walk can be calculated roughly. The mean rate of the walk at Boulder City was about 5.6 km. per hour. On a 5 per cent grade this calls for an oxygen intake of about 0.8 liter per minute going down and 1.7 liter per minute going up. Hence for a walk of 20 miles approximately 430 liters of oxygen must have been used. With a calorific equivalent of 4.9 this corresponds to an expenditure of 2000 kgm.-cals. for walking the 20 miles. The requirement for the remaining 18 hours probably brought the 24-hour total to a value in excess of 3500 kgm.-cals. and possibly to a value as high as 4000 kgm.-cals. This day's work may be rated as medium to heavy. The fact that man can perform so much work at Boulder City indicates that after acclimatization has taken place, an environmental temperature of 40°C. reduces his working capacity but little so long as the humidity is low and the air movement moderate.

The dog, a male fox-terrier, was obtained from a near-by pound on June 15th. Except for frequent walks on the desert, he spent nearly all the summer, day and night, in the laboratory, where the temperature ranged from 30 to 35°. His previous history is unknown but we can say that he had at least as good an opportunity for acclimatization as the members of our party.

A few preliminary observations made it evident that dogs are not well adapted to high temperature, particularly when the sunlight is intense. Left to their own devices they remain inactive during the hottest part of the day. They lie in the shade, if possible on moist ground. Our dog, when taken out on a leash, soon became quite uncomfortable. Notwithstanding continuous panting his rectal temperature always increased, reaching on one occasion 42.3°. He did not take the dog's customary interest in his environment, but walked with tail between legs and with drooping ears. His feet were sometimes blistered by the hot sand. In the final walk several cotton tobacco bags fastened over each foot reduced discomfort from this source.

The final walk at Boulder City was begun 2 hours after breakfast on August 3, and was continued without a pause for lunch. Hour by hour the dog became more uncomfortable and less willing to continue. The difficulty of pulling him along on the fourth round-trip was so great that he was left behind on the last trip.

When the similar experiment was carried out in Boston two months later the dog showed no evidence of fatigue. He panted but slightly, his

After one three-hour walk he returned to the laboratory with a rectal temperature of 42.3° and within a few minutes vomited about 500 cc. of clear fluid.

temperature remained unchanged and his tail and ears were erect. For D. B. D., on the contrary, there was subjectively as much fatigue as at Boulder City.

We may now refer to table 1 which shows the water exchange and rectal temperature in the two experiments. The water intake at Boulder City

TABLE 1

Water exchange and rectal temperature during a walk of 32 kilometers

TIME	REMARKS	DBD			DOG		
		Water intake	Weight	Rectal temperature	Water intake	Weight	Rectal temperature
A. Boulder City at 40 to 42°							
hours		*kgm.*	*kgm.*	*°C.*	*kgm.*	*kgm.*	*°C.*
0	Walk begun		75.0	37.2		16.0	39.7
1.2		1.7	74.5	37.9	0.85	15.9	41.3
2.5		1.1	74.0	38.0	0.52	15.8	41.2
4.2		1.1	73.2	38.0	0.63	15.8	41.5
5.8	Dog stopped (26 km.)	1.1	72.7	38.0	0.45	15.9	41.4
7.1	DBD stopped	1.1	71.9	38.0			
24			73.1			16.0	
B. Boston at 22 to 24°							
0	Walk begun		74.7	37.5		18.9	39.9
1.5		0.25	74.3	37.8	0.08	18.8	39.8
3.0		0.50	73.8	38.2	0.11	18.6	39.5
4.6		0.50	73.7	38.2	0.39	18.5	39.7
6.2		0.25	73.6	38.2	0.10	18.5	39.7
7.5	Walk ended	0.25	73.4	38.2	0.29	18.4	39.1

TABLE 2

Gross and net water loss

	DBD		DOG	
	Boulder City	Boston	Boulder City	Boston
Gross loss, hourly, *per cent of body weight*...	1.74	0.50	2.61	0.97
Gross loss, total, *per cent of body weight*....	12.4	3.7	15.2	7.3
Net loss, hourly, *per cent of body weight*....	0.59	0.23	0.1	0.36
Net loss, total, *per cent of body weight*.....	4.2	1.8	0.7	2.7

was about 1.2 liter per round-trip for D. B. D. and one-half as much for the dog. D. B. D., however, lost 3.1 kgm. in weight while the dog's weight remained nearly constant. The actual water lost per round-trip (correcting for urine output) was 1.8 kgm. for man and one-third as much for the

dog. At Boston the corresponding figures were 0.56 and 0.27. The evaporation in per cent of body weight is shown in table 2.

After discounting somewhat the foregoing estimates for the dog for reasons already mentioned it is probable that at Boulder City the dog evaporated more water per pound of body weight than man. Yet he was less successful in maintaining a comfortable temperature. The explanation of the breakdown of his heat-dissipative system is simple. The temperature of his dry skin approaches that of the air. The temperature of areas exposed to the sun may considerably exceed that of the body with the result that heat is absorbed. On one occasion such an exposed area was found to have a temperature of 46° when the air temperature was 42° and his body temperature, 41.5°. Under such conditions the heat dissipated from the mouth and respiratory system must include not only that produced by chemical processes in tissues but also that absorbed by the skin. Only when the air temperature falls below body temperature can heat be dissipated from the dry body surface, the rate of dissipation by this avenue increasing with decreasing external temperature.

A consistent difference between the response of man and dog during exercise at high temperature is that the dog tends to maintain his weight more nearly constant than man. Again the explanation is simple; man loses much salt in the sweat while the dog loses none except that in the saliva which drops from his mouth. This difference is reflected in certain properties of the blood which are tabulated in table 3.

The concentration of hemoglobin in the blood depends upon several factors. If water is leaving the serum its relative volume decreases and that of the cells increases. The function of the dog's spleen in acting as a labile reservoir for red cells introduces a second variable. Finally there may be destruction of red cells, shown by Broun (4) to be a particularly important phenomenon in dogs. In our experiments there was an increase in the hemoglobin concentration of from 2 to 6 per cent in D. B. D. in each experiment. At Boulder City the concentration had returned to its normal value by the next morning, indicating that no considerable destruction of red cells had occurred. In the dog on the other hand there was a significant decrease in both cases, amounting to 7 per cent at Boulder City and 10 per cent at Boston. Roughly one-half of these decreases can be accounted for by the blood drawn for analysis; whether the remainder is due to red cell destruction, to red cell storage or to fluid transfer cannot now be answered. The further decrease of 15 per cent during the recovery period at Boulder City is even more difficult to explain.

The oxygen combining capacity of cells was calculated from the oxygen capacity of whole blood and the percentage cell volume. The constancy of this value in every case proves that water is not abstracted from the red cells even during excessive sweating.

The carbon dioxide capacity of oxygenated whole blood at a carbon dioxide pressure of 40 mm. is a measure of alkaline reserve. The slight decreases in alkaline reserve were partly due to the accumulation of lactic acid. The increases in lactic acid concentration for the dog were 1.6 m.-eq. at Boulder City and 0.4 at Boston. For man the increase was 1.0 m.-eq.

TABLE 3

Properties of the blood during a walk of 32 kilometers

TIME	DBD						DOG					
	HbO_2 capacity	CO_2 capacity at pCO_2 = 40 mm.	Serum protein	Serum chloride*	rCl*		HbO_2 capacity	CO_2 capacity at pCO_2 = 40 mm.	Serum protein	Serum chloride*	rCl*	
A. Boulder City at 40 to 42°												
hours	*m.-eq. per liter of blood*	*m.-eq. per liter of cells*	*m.-eq. per liter of blood*	*grams per liter of serum*	*m.-eq. per liter of serum*		*m.-eq. per liter of blood*	*m.-eq. per liter of cells*	*m.-eq. per liter of blood*	*grams per liter of serum*	*m.-eq. per liter of serum*	
0	7.8	19.1	21.9	73.5	102.6	0.63	9.6	20.3	18.7	63.5	111.5	0.63
1.2	7.9	19.3	20.7	76.6	105.4		8.8	20.2	16.5	62.8	114.7	0.61
2.5	8.0	19.3	20.5	77.4	101.4	0.62	9.0	20.4	16.2	63.9	113.7	0.62
4.2	8.0	18.5	20.9	79.4	104.7	0.63	9.5	19.7	16.3	64.8	116.8	0.63
5.8	8.2	18.2	20.0	83.7	102.3	0.65	8.9	19.3	16.3	63.2	113.2	0.64
7.1	8.4	19.1	19.8	87.9	103.5	0.64						
24	7.9	18.7	20.5	80.1	102.8	0.60	7.5	20.2	18.8	60.0	115.8	0.63
B. Boston at 22 to 24°												
0	8.0	18.5	20.8	76.8	108.3	0.63	10.1	19.5	20.0	64.9	114.5	0.66
1.5	8.3	18.9	21.6	75.8	107.9	0.65	9.9	20.1	19.0	69.8	115.5	0.66
3.0	8.6	19.6	20.8	77.8	107.9	0.64	9.8	20.5	18.4	63.6	116.5	0.66
4.6	8.4	19.2	20.0	78.1	106.9	0.65	9.2	20.3	18.8	67.6	116.3	0.67
6.2	8.4	19.7	19.3	83.8	104.1	0.66	9.1	20.1	18.2	63.6	115.6	0.66
7.5	8.3	18.8	19.3	82.9	103.7	0.64	9.0	19.9	18.2	63.3	114.8	

* The values given in this table for serum chloride and rCl, i.e., $\frac{\text{cell chloride}}{\text{cell water}} \div \frac{\text{serum chloride}}{\text{serum water}}$, have been calculated to a pH of 7.45. The experimental determination of serum chloride, cell chloride, serum water and cell water were made at a pH value near 7.45. The data of Dill, Edwards, Florkin and Campbell furnish the basis for recalculation (Journ. Biol. Chem., 1932, xcv, 143).

in each case. These increases account for one-half the decrease in alkaline reserve; the remainder is of little consequence. The fact that little lactic acid accumulated in the blood is due to the moderate rate of oxygen intake. It has been shown in previous communications (5), (6) that an oxygen requirement of 1.7 liter per minute results in little lactic acid

accumulation in a well-trained man. There was more lactic acid in the dog at Boulder City than in any other case; this is related to his high temperature as has been shown by Dill, Talbott and Edwards (3).

The constancy of the serum chloride concentration in the Boulder City experiments is of some interest. Water was taken *ad libitum* by man and dog, associated in one case with decreasing body weight and in the other with constant weight. The difference in response is significant and will be referred to below. The constancy in man and dog of the serum chloride concentration suggests a close relationship between it and the phenomenon of thirst.

The serum protein concentration has been found to give the most useful measure of net water loss as appears in figure 1. The relation is uniform;

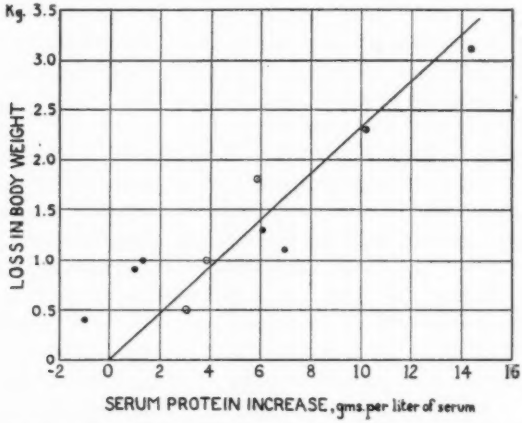

Fig. 1. Increase in serum protein concentration in relation to loss in body weight. Hollow circles, Boulder City; solid circles, Boston.

if we assume that for D. B. D. the loss of 1 kgm. in weight was associated with an increase in serum protein concentration of 4.3 grams per liter, the differences between calculated and observed body weights in the Boulder City experiment are -0.22, $+0.10$, $+0.43$, -0.07 and -0.23 kgm. The agreement in the Boston experiment is not so good but the weight changes were less so that second-order phenomena became more important.

The differences between man and dog are evident. Man loses weight while the dog maintains his weight. The mechanism of such a weight loss in man was shown by Cohnheim, Kreglinger and Kreglinger (7) to be due to loss of salt. The original weight is regained only when the salt is restored. It has been shown by Talbott and Michelsen (8) that this characteristic of the heat-dissipative system of man may lead to serious

consequences when there is an inadequate salt intake with the food. In one respect, therefore, the dog is superior to man because he loses but little salt and is able to equalize water intake and output.

The dog is superior to man in another respect. It has been shown by Steinhaus (9) that the dog may maintain an arterial carbon dioxide pressure of 10 mm. or less during work in a hot environment. Thus he is capable of a much greater alveolar ventilation than man in exercise and a larger proportion of heat will be dissipated from his lungs than in man. This is an economy since dissipation of heat from the lungs places no additional burden on the circulation. Heat dissipated from the skin in man requires a shunt in the circulation that immobilizes part of the blood stream insofar as its gas-transport function is concerned.

The dog possesses an additional advantage which is of great importance in the laboratory where there may be no air movement. He supplies his own air circulation over the significant areas. His superiority over man in dissipating the heat produced in exercise under ordinary laboratory conditions is related to this fact. Man doing hard work in the laboratory requires air movement for comfort.

Notwithstanding these advantages the dog proved inferior to man at Boulder City. His dry skin acquired a temperature above body temperature, placing an added burden upon his heat-dissipative mechanism. The air movement, together with the low humidity, kept the skin of man cool, while air movement was disadvantageous to the dog since it favored absorption of heat through the skin. It is not possible to go much further in discussing his breakdown at Boulder City. His body temperature was uncomfortably high but it is possible that his skin temperature may have caused him more acute discomfort than his body temperature. His work output was less than one-third his capacity at low temperature. On one occasion, after his return to Boston, he ran on a 17.6 per cent grade at 7.4 km. per hour for 7.5 hours with a 5-minute stop each half-hour for water. In this case with the room temperature varying from 14 to 17° his rectal temperature varied from 40.5 to 41.1°. He was not exhausted at the end although the total energy output was 3 times that of the Boulder City walk.

SUMMARY

Although superior to man in dissipating heat in the laboratory, the dog is inferior in the hot, arid desert. Under environmental conditions which permit man to dissipate heat from his entire body surface, the dog is absorbing heat through his dry skin. Modifications in the properties of the blood during a long walk are subject to simple interpretations. Man loses salt in his sweat and undergoes a decrease in body weight associated with an increase in serum protein concentration when water is drunk *ad libitum*.

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OVARIAN RESPONSES TO PROLAN AND ANTERIOR PITUITARY EXTRACT IN HYPOPHYSECTOMIZED RABBITS WITH PARTICULAR REFERENCE TO OVULATION[1]

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Work thus far reported has not given information upon the question as to whether or not minimal ovulating doses of anterior pituitary extracts and of prolan must necessarily be increased in the rabbit following hypophysectomy. Although it is known that large doses will stimulate ovulation in the absence of the hypophysis, experiments with known doses of hormone upon the normal and hypophysectomized animals could be interpreted as indicating a stimulation of the intact pituitary by these extracts. The use of hypophysectomized animals in such studies is essential, for the secretion of the animal's own gland introduces an unknown factor. The rabbit as an experimental form has a unique advantage in studies of this nature because the time of ovulation can be predicted with great precision. Suggestive data have been secured with regard to the above question in the following experiments. Data are also presented on the structural and functional development of the corpora lutea after hypophysectomy and upon the capacity of extracts of the anterior hypophysis and prolan to induce follicular growth in hypophysectomized animals.

Hill and Parkes (1931) in an early work showed that a pituitary extract given intravenously was quite capable of inducing ovulation in the hypophysectomized (decerebrate) rabbit and that prolan (from pregnancy urine) was usually incapable of doing so. Later Hill and Parkes (1931) reversed their view and showed that prolan was just as effective as the gland extract in producing ovulation. In neither case were minimal ovulating doses employed. Kraul (1932) and Reichert (1932) reported that it was nearly impossible or very difficult to induce follicular development in hypophysectomized rats by the injections of prolan. Recently it was shown by one of us (Leonard, 1932) that a larger dose of prolan in terms

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of rat units was required to induce ovulation in the rabbit than if anterior pituitary extracts were used. This suggested the possibility that perhaps small ovulating doses of prolan would be incapable of inducing ovulation in the hypophysectomized animal.

The technique of hypophysectomy was the same as reported previously by Smith and White (1931) in which the buccal approach was used. Complete ablation of the pituitary was verified by histological examination of the capsule after autopsy of the animal. The rabbits were kept isolated for twenty days before being operated upon and only those of the isolated group were used which showed unmistakable signs of heat by an enlarged purplish vulva. After hypophysectomy, they were injected intravenously with the various doses of the extracts which were previously standardized on normal rabbits. Since the operation requires approximately 1 hour, all injections were made $1\frac{3}{4}$ hour after the beginning of the operation, the animal thus being without its hypophysis approximately 1 hour before injection. Exploratory laparotomies were performed 15 to 20 hours later to determine whether ovulation had occurred.

The extract of pregnancy urine (prolan) used was a commercial preparation, "Follutein,"[4] standardized to 50 mouse units per ccm. On restandardization in rat units it was found that approximately 1 mouse unit equalled 2 rat units. The unit chosen here is that described by Zondek (1931). The extract of anterior pituitary used was the water soluble fraction of a pyridine extract of sheep gland prepared by the method of Fevold, Hisaw and Leonard (1931).[5] The same preparation was used throughout the series of experiments.

Table 1 shows the standardization of the anterior lobe extract and prolan on normal rabbits. For the pituitary extract, the M.O.D. (minimal ovulating dose) was 0.015 gram of dried sheep gland, corresponding to $\frac{1}{10}$ rat unit of the extract used. For prolan, the M.O.D. was 2 rat units (1 mouse unit). This latter result coincides very well with the recent findings of Friedman (1932) who reports the M.O.D. for the rabbit to be 1 rat unit per kilogram of body weight.

Ovulation in the hypophysectomized rabbits. The follicles in the ovaries

[4] The "Follutein" was furnished to us through the kindness of Doctor Morrell of E. R. Squibb & Co.

[5] The sheep glands were kindly furnished by Parke, Davis & Co. The standardization of the gonadotropic hormones of the pituitary is quite unsatisfactory with immature rats and mice. Any one or a combination of the three Zondek reactions may be obtained with a uniform dose in littermate animals. It has often been our experience that an immature ovary can be made to respond physiologically without any definite morphological changes being visible. For the sake of comparison, a unit here can be considered as the smallest dose when given twice daily for 5 days to produce definite lutein tissue in an immature rat ovary. The particular extract used was found to be 1 rat unit equal to 0.15 gram of powdered gland.

of hypophysectomized rabbits undergo a marked and rapid involution. It is desirable, then, to inject the ovulation producing substances as quickly as possible to eliminate any untoward changes in the ripe follicles to be stimulated. When the anterior pituitary extract was injected into the operated animal, it was found to require from 0.0175 to 0.02 gram of gland to invariably produce ovulation, which is about $\frac{1}{6}$ to $\frac{1}{3}$ of an M.O.D. more than the control (table 1). Similarly, the ovulating dose of prolan (2 R.U.) had to be increased to 3 units or about $\frac{1}{2}$ of an M.O.D. more in the hypophysectomized animal (table 1). An animal subjected to ether anesthesia for the same period of time as the duration of the operation did not require more extract to induce ovulation. Whether the trauma of the operation or the retrogressive changes in the follicle that might take

TABLE 1

Determination of minimal ovulating dose (M. O. D.) of prolan and sheep anterior pituitary extract in normal and hypophysectomized rabbits

NORMAL RABBITS			HYPOPHYSECTOMIZED RABBITS		
Number of rabbits	Dose of A. P. extract	Ovulation	Number of rabbits	Dose of A. P. extract	Ovulation
	grams			*grams*	
6	0.005–0.0125	6–	4	0.015	3– 1+
4	0.015–0.02	4+	4	0.0175–0.02	4+
M. O. D. = 0.015 gram			M. O. D. = 0.0175–0.02 gram		
	Dose of prolan			Dose of prolan	
6	1–1.5 R. U.	5– 1+	4	2–2.6 R. U.	4–
4	2–10 R. U.	4+	3	3 R. U.	3+
M. O. D. = 2 R. U.			M. O. D. = 3 R. U.		

place within an hour after removing the pituitary and before the injections caused the increased demand for the ovulating principle is not known. It was noted that a greater increase in the M.O.D. of prolan over anterior pituitary extract was necessary to induce ovulation in the hypophysectomized rabbit.

Fate of the corpora lutea formed. Deansly, Fee and Parkes (1931) reported that if a rabbit is bred and has its hypophysis removed $1\frac{1}{2}$ hour or longer after mating, ovulation will take place normally, and corpora lutea will form. Because of their technique, the animals could not survive and were only in a sufficiently healthy condition to be usable for 36 hours. The corpora had thus developed for approximately 26 hours. Later, Smith and White (1931), using a different technique, confirmed these findings

and demonstrated that the corpora on the 4th and 8th days were decidedly smaller than those of the controls. The corpora resulting from the ovulations induced by extracts in the hypophysectomized rabbits were similar to those reported by Smith and White.

These lutein bodies were found to be functioning sufficiently, however, to produce a good pseudopregnant endometrium in the uterus of the rabbit (see fig. 1). By the 8th day, the uterus of these animals had become similar to that of a castrate and the corpora had almost completely degenerated. Physiologically, these small corpora lutea are not equal to those of the normal controls.

Follicular stimulation with extracts in hypophysectomized rabbits. From the work on normal rabbits (Leonard, 1932) in which it was demonstrated that it required larger amounts of prolan than pituitary extract to induce

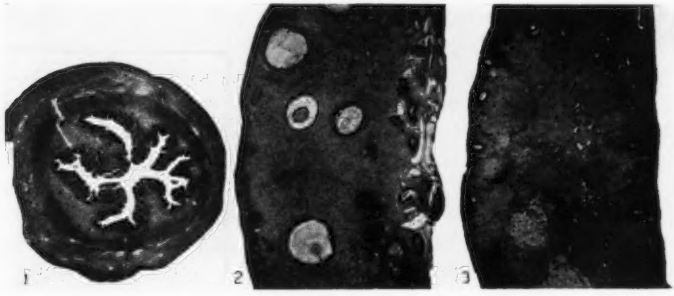

Fig. 1. Progestational uterine changes demonstrating that the corpora lutea formed in the absence of the hypophysis are producing some lutein hormone.

Fig. 2. Follicles in the ovary of the hypophysectomized rabbit produced by 83 ovulating units of pituitary extract.

Fig. 3. Ovary of hypophysectomized rabbit injected with 250 ovulating units of prolan. Note the absence of follicles.

ovulation, the question arose whether or not it would require larger doses of prolan to produce new follicles in the ovaries of rabbits hypophysectomized sufficiently long for the old follicles to have degenerated. This work is not completed as yet but nevertheless the results obtained appear significant. Subcutaneous injections totaling 83 M.O.D. of the sheep anterior lobe extract were made into rabbits hypophysectomized 1, 4, 6, and 29 days previously. The treatment was continued for 5 days and the animals were killed on the 7th day. In all cases definite follicular stimulation resulted as indicated by the appearance of numerous medium sized follicles and cystic follicles (see fig. 2). In this, the first attempt, insufficient amounts were given for too short a period to cause any perceptible growth in the uterus, particularly in the animal hypophysectomized for

29 days, when the greatest amount of atrophy had occurred. The injections were not continued sufficiently long to determine if corpora lutea would form.

However, by contrasting these four rabbits with three others that received 100, 100, 250 M.O.D. of prolan and another which received an extract equivalent to 300 cc. of pregnancy urine (estimated potency of 600 M.O.D.), it was seen that no stimulation of follicular growth occurred except in the last case when it was only to a slight degree. These animals were hypophysectomized for 6, 6, 1, and 4 days respectively. By the 6th day after hypophysectomy with no subsequent treatment, no follicles with large antra were present. Yet in spite of the injections of 100 to 600 ovulating units, no new follicles were formed although it appeared in the ovaries of the animal receiving the largest dose that some stimulation occurred. Thus prolan is much less effective than anterior pituitary extract in producing follicular growth in the hypophysectomized rabbit (cf. figs. 2 and 3).

Discussion. The question whether or not the animal's own gland plays a part when ovulation is induced by the injection of prolan or anterior pituitary extract is difficult to determine from the data given above. That it plays at most but a small part is evident. It readily can be conceived that at any given instant there is a definite concentration of the anterior pituitary hormone circulating in the blood which is maintaining the ripe follicles in the ovary. They remain in such a state until there is a sudden increase in hormone concentration of the blood (produced by either copulation or injection) which precipitates ovulation. If the injected hormone set in motion some mechanism whereby a release of the ovulating principle in the animal's own gland brought about a result, then it would seem that the stimulating substance would not react so readily when given in the absence of the hypophysis. It required only $\frac{1}{6}$ to $\frac{1}{3}$ of the M.O.D. of pituitary extract or $\frac{1}{2}$ of the M.O.D. of prolan more to induce ovulation in the hypophysectomized than in the normal animal. When one considers that the gland had been removed 1 hour before the stimulating dose could be given and that the animal had received some trauma, it seems that a slightly increased dosage would be necessary.

It was seen that in the normal rabbit, the substance from pregnancy urine that induces ovulation is required in larger amounts than pituitary extracts when the standard of comparison is the immature rat. Also the increase in the minimal ovulating dose of prolan was found to be 12 per cent to 29 per cent more than that of the anterior pituitary hormone in the hypophysectomized rabbits. Furthermore, this difference in the effective dosage of the two extracts is again made evident when the follicles have undergone atresia due to the removal of the pituitary and the ineffectiveness of prolan becomes then even more pronounced. This relative

ineffectiveness of prolan has been demonstrated by Reichert et al. (1932) for the production of follicles in the hypophysectomized rat.

Such results as presented show a physiological difference between prolan and the gonadotropic hormones of the pituitary but as yet there are insufficient data to explain the physiological difference in their reactions. Two explanations have been put forth, one by Evans and his co-workers and the other by Kraul. Evans et al. (1932) believe that prolan converts the growth hormone in the normal gland into the sex hormone. Thus in the absence of the hypophysis, prolan would be ineffective since it has no material upon which to react. On the other hand Kraul (1932) believes that prolan contains a very large proportion of the luteinizing hormone so that in the absence of a large follicle prolan is relatively ineffective.

It was interesting to discover as mentioned above that these small corpora lutea which formed after ovulation in the hypophysectomized rabbit were functional whether produced by prolan or anterior pituitary extract. In four cases examined, a definite progestational modification was produced. That some pituitary hormone essential for the development of the corpora lutea was lacking is evident because the lutein bodies were grossly distinguished as being much smaller than normal. It may be added that physiologically these corpora are not producing their maximum amount of hormone as indicated by the degree of glandular development in the uterine endometrium.

CONCLUSIONS

1. It takes $\frac{1}{6}$ to $\frac{1}{3}$ more of a minimal ovulating dose of an anterior lobe extract and $\frac{1}{2}$ more of a minimal ovulating dose of prolan to induce ovulation in a rabbit hypophysectomized in heat.

2. It would seem that in the experimental animal the injected hormone producing ovulation was acting independently of the intact hypophysis else a greater increase in the injected hormone would be necessary.

3. The corpora lutea that form from such methods of stimulation in the hypophysectomized rabbit are similar to those previously reported by Smith and White. These smaller corpora are quite capable, however, of producing sufficient corpus luteum hormone to form, histologically, a progestational endometrium in the uterus.

4. In order to induce follicular stimulation in the ovaries of hypophysectomized rabbits, a great increase of prolan over gonadotropic pituitary preparations is needed.

We wish to express our appreciation to Dr. Philip E. Smith for his helpful criticism of this work.

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STUDIES OF CEREBRAL FUNCTION IN LEARNING

X. The Effects of Dilatation of the Ventricles upon Maze-Learning

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The interpretation of symptoms following local destruction of tissue in the brain is complicated by the possibility of various circulatory and trophic disturbances within the remaining tissue, whose effects upon behavior cannot be evaluated from our present knowledge. In addition to the symptoms which are characteristic only of lesions within specific areas, such as scotomas from injuries to the area striata, there appear defects which are essentially the same after lesions in anatomically diverse cortical areas. Such, for example, are deficiencies in attention, memory, or intelligence and alterations of personality. It has not been possible to correlate these symptoms with lesions in specific loci, and the majority of investigators have ascribed them to a metabolic disturbance of the tissue not directly involved in the lesion. Thus Piéron (1927) says, "Such symptoms are not connected . . . with a limited destruction of the brain whereever this may occur, but with a disturbance of cellular activity as a whole by disturbance of neuronic metabolism." Pavlov (1927) has stressed the disorders resulting from the tension and pressure exerted by scar tissue and seems inclined to ascribe the non-localizable symptoms to such causes. Buytendijk (1932) lists circulatory disturbances and dilatation of the ventricles among other possible sources of error in studies of cerebral localization.

In several studies of cerebral function in the rat the senior author has reported a close correlation between the extent of cerebral destruction and the severity of deterioration of certain functions, irrespective of the locus of the lesion.[1] Since in such cases other functions may remain undisturbed, even when their specific cortical fields are invaded by the lesions,[2] he ascribed the defects to the absence of the tissue destroyed rather than to metabolic disturbance in that remaining. From this it was inferred

[1] For a summary of the various studies dealing with this problem see Lashley (1933).

[2] As when the discrimination of visual patterns persists after lesions which invade the striate area and which seriously disturb maze-learning.

that, in addition to its specific integrative functions, each cortical field contributes to the general mass of cortical excitation upon which the excitability and efficiency of the whole depends (Lashley, 1932). Piéron has made a similar suggestion (1927, p. 53) but regards the loss of tissue as of slight importance for the production of non-focalized symptoms in comparison with the metabolic disturbances.

A crucial test of the relative importance of trophic disorders and the removal of nervous tissue is difficult to devise, since on the one hand disorders of metabolism are likely to result in diffuse degeneration of neurons with actual reduction in the quantity of nervous tissue, and on the other, general metabolic disturbance may be suspected after any localized lesion. The major causes of metabolic disturbance in the brain tissues following operations on the cerebrum are probably vascular changes from pressure or shock, changes in the fluid contents of the tissues resulting from pressure, local irritation, or excessive production of cerebro-spinal fluid, and chemical influences due to the absorption of necrotic tissue or clots.

The present experiments were designed to determine whether or not increased intracranial pressure with the consequent vascular disturbances and distortion of tissues might have been responsible for the non-localizable defects in learning ability previously reported in studies with the maze. The problem has been approached in two ways: first, by compression of the brain through the injection of an inert substance into the ventricles with subsequent training of the animals in the maze, and second, by comparison of the rates of learning after destruction of parts of the cerebral cortex in animals with and without an accompanying hydrocephalus.

MECHANICAL DILATATION OF THE VENTRICLES. *Animals.* Adult male rats, 150 to 200 days of age, from the strain previously used by Lashley and Wiley (1933), were selected for the experiments.

Dilatation of the ventricles. With the animal under deep ether anesthesia, a 2 mm. trephine hole was made in the middle of the left parietal bone. Through this opening a fine hypodermic needle was passed into the right lateral ventricle, the deep insertion being necessary to prevent leakage of the injection fluid around the needle. Sterile paraffine oil at body temperature was injected into the ventricles until respiration stopped. The needle was then withdrawn and the wound closed. Respiration was usually resumed spontaneously within 15 to 30 seconds. In this way from 200 to 400 cu. mm. of oil were introduced, some of which later escaped through the needle puncture. Twenty-three animals survived for tests of learning. As a check upon the effects of the oil, physiological salt solution was injected instead of oil in five additional animals.

Following the injection all of the animals showed marked symptoms of high intracranial pressure; labored breathing, paralysis or spasticity of the

limbs, opisthotonus or a tendency to rotate to one side. These symptoms cleared up within a few hours, leaving the animals normal to casual inspection. The wounds healed promptly and at the end of two weeks after operation, when training was begun, the animals were in good physical condition.

Training. The 8 cul de sac maze previously used by Lashley for studies of the relation between extent of cortical destruction and maze-learning ability was used in our experiments. The animals were given 5 trials per day until 10 consecutive errorless runs were obtained. Errors were recorded automatically. The general procedure was as previously described (Lashley, 1929, maze III).

No normal controls were trained, since we already have records of the training of many normal animals in this maze and since comparison of different animals within our operated group seemed likely to be more significant than a check against a normal series.

Determination of lesions. At the conclusion of training the animals were killed and the brains hardened *in situ* in 10 per cent formalin. After hardening the brains were removed, sectioned, and stained with iron hematoxylin and neutral red. Camera drawings of serial sections at intervals of 0.25 mm. through each brain were made. The surface areas of the ventricles and of the tissue in each section were measured on the drawings with a planimeter and from these areas and the distance of separation of the sections the volumes of the ventricles and of the brain tissues were computed. Since the brains differed considerably in size, the volume of the ventricles was finally expressed in each case as percentage of the volume of tissue. For comparison, this ratio was determined also for five normal brains fixed and sectioned by the same method.

The lesions to the cortex resulting from the needle punctures were reconstructed by the graphic method, their areas measured, and expressed as percentage of the surface area of the neopallium.[3]

The average volume of the ventricles of the normal brains was 0.26 per cent of the volume of nervous tissue or, in absolute terms, about 1.9 cu. mm. The maximum dilatation of the ventricles produced by injection of oil was to 15.7 per cent of the volume of nervous tissue or 118.7 cu. mm. This is more than 60 times the normal volume. The average dilatation for the 23 injected cases was to 6.8 per cent of the volume of tissue, to 26 times the normal volume.

The chief alteration was in the lateral ventricles. In a few cases (table 1) the third ventricle was enlarged and in others the aqueduct was dilated,

[3] The correlations between extent of cortical destruction and maze records have been computed for our series. They are: for trials, $r = 0.19 \pm 0.28$; for errors, $r = 0.23 \pm 0.28$. As was expected from earlier work, there is no significant relation when the range of lesions is so small.

with apparently some changes in the fourth ventricle. Figure 1 shows the condition of the ventricles in a normal brain and in brains with average (6.7 per cent) and maximum (15.7 per cent) dilatation.

The question arises whether the dilatation was due solely to the mechanical pressure of the oil injected or to a genuine hydrocephalus resulting from the presence of the foreign substance in the ventricles. The volume of the

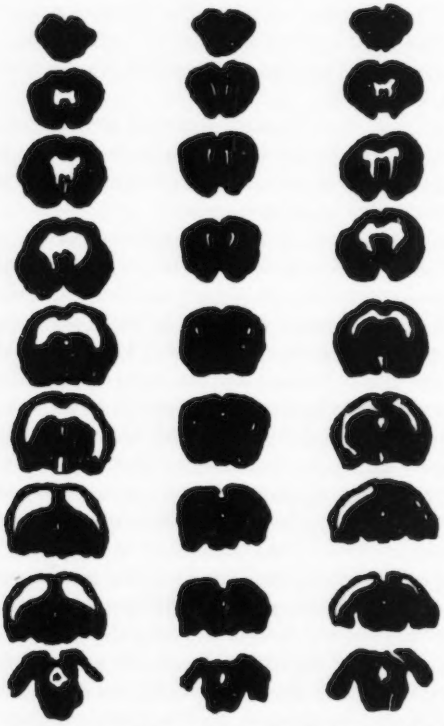

Fig. 1. Camera sketches of sections at intervals of about 1.50 mm. through three brains to show the proportionate size of the ventricles. The middle series is from a normal animal, that on the left from case number 23 with the volume of the ventricles 15.7 per cent of the volume of the tissue, that on the right from number 14 with dilatation to 6.7 per cent, which is approximately the average for the group.

ventricles in no case equaled the volume of oil injected. In case number 23, with the greatest dilatation, the volume of the ventricles was 118.7 cu. mm. after injection of 300 cu. mm. of oil. In two cases with marked dilatation the ventricles were opened before the brains were dehydrated. The spaces seemed to be completely filled with oil. In sections the ependyma and plexuses showed no trace of the thickening described for

cases of hydrocephalus in man. This evidence indicates that the dilatation was a direct mechanical effect of the oil and not the result of a secondarily induced hydrocephalus.

TABLE 1

Records of animals trained in the 8 cul de sac maze after injection of oil (numbers 1 to 23) or physiological saline (numbers 24 to 28)

The volume of the ventricles is expressed as percentage of the volume of brain tissue, the injury to the cortex as percentage of the surface area of the neopallium.

NUMBER	VOLUME OF VENTRICLES	TRAINING		ERRORS LESS FIRST TRIAL	LESION TO CORTEX	THIRD VENTRICLE ENLARGED	CORTEX PIERCED	AQUEDUCT CLOSED
		Trials	Errors					
1	0.26	25	28	23	0.40	−	−	−
2	0.62	31	40	37	0.46	−	−	−
3	2.52	66	110	107	3.20	−	+	−
4	2.86	40	164	140	0.94	−	+	−
5	2.88	62	43	31	4.57	−	+	−
6	3.02	74	197	177	1.32	+	+	+?
7	3.26	50	76	58	1.32	−	−	−
8	3.86	93	218	215	1.96	−	+	−
9	4.17	56	93	78	0.74	+	−	−
10	4.83	18	28	19	2.64	−	+	+
11	5.41	55	68	61	3.01	−	+	−
12	5.89	55	155	127	5.09	−	+	−
13	6.66	39	68	63	2.26	+	−	+
14	6.70	59	46	42	1.13	−	+	−
15	7.19	47	94	69	2.50	−	+	−
16	8.12	114	113	106	2.64	−	+	−
17	8.80	71	333	328	3.39	+	+	−
18	9.47	59	221	207	0.75	+	−	+
19	9.55	30	29	21	1.88	−	+	−
20	14.05	98	129	125	1.13	+	−	−
21	14.09	69	101	92	0.62	−	−	−
22	15.73	27	38	33	0.56	−	−	−
23	15.75	68	70	68	0.30	+	−	−
Average...	6.76	56.9	107.4	96.8				
P.E.....	0.58	3.3	10.3	10.5				
24	0.18	103	174	151	0.43	−	−	−
25	0.19	35	145	130	0.22	−	−	−
26	0.44	65	282	260	1.03	−	−	−
27	0.59	73	82	77	0.62	−	−	−
28	2.51	35	70	67	0.30	−	−	−
Average...	0.78	62.2	150.6	137.0				
P.E.....	0.26	7.72	22.8	20.8				

Is the enlargement of the ventricles attended by a shrinkage of the nervous tissue? Only indirect evidence on this point is available. Since the normal volume of the ventricles is insignificant in relation to the volume of

the brain, and since the amount of dilatation was in each case a chance result of the position of the injecting needle, no correlation is to be expected between normal brain volume and the degree of dilatation of the ventricles. Therefore, if enlargement of the ventricles took place at the expense of nervous tissue, there should be a significant negative correlation between the volume of the ventricles and that of the tissue. The correlation between these variables was computed. It is $r = 0.118 \pm 0.145$, showing that there is no relationship between the variables. The enlargement of the ventricles must have been compensated for by absorption of fluid from the subarachnoid space. The character of the distortion of the brains bears out this interpretation.

Analysis of data. The individual training records of the animals with brief statements of the condition of the cortex and ventricles are given in table 1. Numbers 1 to 23 received injections of oil, numbers 24 to 28 physiological saline. We may test the effects of dilatation of the ventricles in three ways: 1, by comparison of these two groups; 2, by comparison of the oil group with the records of normal animals trained in the same maze or with the records of animals having different amounts of cortical destruction; 3, by correlating the size of the ventricles among members of the oil group with the individual records in the maze.

1. The animals injected with saline showed practically no enlargement of the ventricles, number 28 alone exceeding the range of normal animals. The small number of animals in the group makes the reliability of the averages uncertain, but there is certainly no indication of superiority to the oil group. The best score of the saline group, that of number 28, is not as good as that of number 22 with 15.7 per cent dilatation, and the average scores are higher than those of the oil group.

2. Lashley (1929) reported the records for two groups of normal animals trained in the same maze used in our experiments. Lashley and Wiley (1933) gave data for another group with initial training in a similar maze and for four groups trained in our maze, after previous training in other mazes. The records for these normal groups were the following:

NUMBER OF CASES	TRIALS	ERRORS	ERRORS LESS FIRST TRIAL	
Normal groups				
22	19.0±1.0	47.0±2.0		Lashley, 1929
59	24.8±1.5	54.5±2.8		Lashley, 1929
15	33.8±1.9		66.7±7.3	Lashley and Wiley, 1933
15	28.7±1.9		44.4±6.3	Lashley and Wiley, 1933
15	25.0±1.5		26.4±3.7	Lashley and Wiley, 1933
15	29.0±3.9		22.9±3.5	Lashley and Wiley, 1933
15	24.8±3.0		38.8±14.6	Lashley and Wiley, 1933
Oil group				
23	56.9±3.3	107.4±10.3	96.8±10.5	

There is a good bit of variation among the normal scores representing chance differences in the groups and in methods of training, but all scores are significantly better than those of the animals in our present experiments. All of our cases have some injury to the cortex and in a few there is damage to the septum and right caudate nucleus. The above comparison does not take into account the cortical injury or give any index of the significance of the inferiority of our cases. This may be gained by comparing them with cases having different amounts of cerebral destruction.

Lashley (1929) has computed the amount of retardation in learning the maze following the destruction of different amounts of cortex, by class intervals of 10 per cent, as follows:

Percentage of neopallium destroyed	1–10	11–20	21–30	31–40	41–50	50+
Errors in learning	72	266	396	485	580	1446

The average of the group with injection of oil, 107 errors, falls between the first and second class intervals. It is somewhat worse than the minimal effect of cerebral lesion, but significantly less than the disturbance produced by a lesion of 10 to 20 per cent of the neocortex. The average destruction of cortex in our cases is 1.9 per cent of the neopallium, but this figure includes only the obvious destruction in the region of the puncture and no account is taken of degenerations in other regions due to rupture of the external capsule. The deterioration following the injection of oil seems little, if any, greater than would be expected from the actual cortical destruction.

3. The most significant test of the effects of dilating the ventricles may be made by comparing the training records of animals with different amounts of dilatation. The range in size of ventricles is from 0.26 to 15.75 per cent of the volume of brain tissue. Correlations (Pearson r) have been computed for trials and for errors with the volume of the ventricles. These constants are:

For trials..... $r = 0.25 \pm 0.09$
For errors..... $r = 0.05 \pm 0.10$

In comparison with the correlations found between extent of cortical destruction and retardation in learning these coefficients are negligible.[4] They give no evidence of any relation between the degree of distention of the ventricles and the retardation in maze-learning.

The various methods of evaluating the data on learning after injection

[4] In studies of the effects of the extent of cerebral destruction upon learning and retention the coefficients of correlation have shown a definite bimodal distribution, few falling between 0.10 and 0.55. In previous discussions a coefficient of 0.70 has been the minimal accepted as clear evidence of a significant relationship.

of oil all point to the same conclusion, that the mechanical distention of the ventricles with the accompanying stretching and distortion of the brain tissues has little, if any effect upon the capacity of the animal to learn, as compared with the marked deterioration following the destruction of cortical tissue. The injection of the oil probably produces only a temporary increase in intracranial pressure. To judge from motor symptoms and the condition of the brains at necropsy, the history of the cases is most probably as follows: at first a marked increase in intracranial pressure resulting from the forcing in of the oil with expansion of the brain to fill the spaces drained by trephining and with severe anemia lasting for a few hours: a rapid adsorption of cerebro-spinal fluid with reduction of intracranial pressure to normal and resumption of normal circulation; dilatation of the ventricles maintained by the oil with final permanent distortion of the tissues and relief from all tension. The residual condition in these cases was therefore probably one of normal pressure with pronounced displacement and distortion of the tissues and serves as a control of these mechanical factors in postoperative deterioration. The possible effects of continued high intracranial pressure require other controls.

THE EFFECTS UPON MAZE-LEARNING OF HYDROCEPHALUS AFTER CORTICAL LESIONS. In experiments involving surgical destruction of parts of the cerebral cortex in the rat the ventricles are frequently pierced and a few of these animals develop a mild degree of hydrocephalus. Lashley and Wiley (1933) have recently reported a series of 127 animals trained in the same maze which we have used, after the destruction of various amounts of cortical tissue. We have reëxamined the sections of the brains of these animals and find dilatation of the ventricles in 22 cases. The maximum distention does not exceed the average of our oil series (6.8 per cent of the volume of the tissue). We have tested the influence of the hydrocephalus first, by comparing the training records of animals with dilated ventricles with the records of others having an equal amount of cortical destruction without evidence of hydrocephalus and, second, by comparing the records of animals with different degrees of dilatation of the ventricles.

Lashley and Wiley give training records for their animals, averaged by class intervals of 10 per cent cortical destruction. We have grouped the cases with dilated ventricles in the same way and computed the average for each class. These averages (for errors exclusive of the first trial made during training, which is the most reliable measure of individual differences) are compared in table 2.

In four of the five classes the hydrocephalic animals are superior to the entire group and in the fifth class (41–50 per cent) their inferiority is largely due to the inclusion of one case with an exceptionally poor record, a score almost double that of any other of the 127 animals, and a very slight degree of hydrocephalus. Because of the small number of cases in

each class of the hydrocephalic animals their deviations from the records of the entire group are not statistically reliable. We may estimate the significance of the differences, however, from the consistency of the data in another respect. Figure 2 respresents graphically the data of table 2 and

TABLE 2

Average scores of animals grouped by class intervals of 10 per cent cortical destruction for 127 rats with cerebral lesions and for the 22 cases from the series in which there were anatomical evidences of hydrocephalus

	PER CENT DESTRUCTION					
	1–10	11–20	21–30	31–40	41–50	51–60
Errors, all cases	52	142	292	499	963	950
Errors, hydrocephalic group	51	77	120	486	1582	

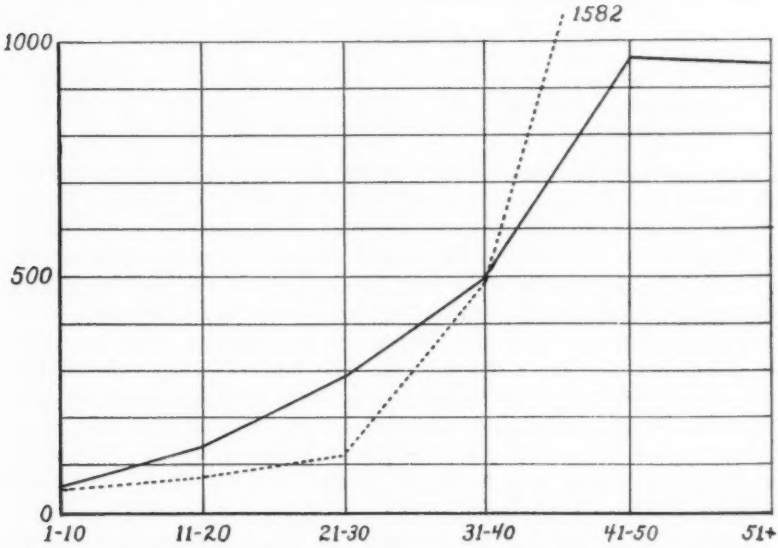

Fig. 2. Graphic presentation of the data of table 2. The ordinates represent percentage of the cortex destroyed, the abscissae the number of errors during maze-learning. ———, averages for a group of 127 animals. - - - -, averages of 22 animals with hydrocephalic dilatation of the ventricles, selected from the total group.

brings out clearly the fact that the learning scores show a constant relation to the extent of cortical destruction. This is true, not only for the large group of 127 cases but also for the smaller group of hydrocephalic animals. From this it is clear that the reliability of the data from the

smaller group is sufficient to give consistent results in relation to the magnitude of the cortical lesion and that the hydrocephalus exerts no such constant influence upon the learning records as does the magnitude of the lesion. The conclusion follows that the relationship between the extent of lesion and the severity of non-focalizable symptoms, shown in this and other experiments (Lashley, 1933) cannot be ascribed to any metabolic changes in the intact tissue arising from the condition of hydrocephalus.

As a further test, the cases with indications of hydrocephalus were divided into two groups, one with less than average, the other with greater than average distention of the ventricles. We could not apply the methods of measurement used with the oil group because in many cases large areas of the cortex had been destroyed and the distended ventricles were continuous with these gaps, so the classification was made by inspection. Though rough, it certainly divided the group into parts with significant differences in the average volume of the ventricles.

The averages for the two groups were the following:

	With lesser dilatation	*With greater dilatation*
Percentage of cortex destroyed	24.9	28.6
Errors in learning	488.7	476.1

The differences between these groups are not significant and the similarity shows, as did our experiment with injection of oil, that learning capacity for the maze is independent of the degree of dilatation of the ventricles.

DISCUSSION. A number of independent studies of learning and retention in various activities after destruction of parts of the rat's cerebral cortex have given evidence for a significant relation between the amount of cerebral tissue destroyed and later efficiency of performance. The reduction in efficiency is, in general, independent of the locus of injury, either within the entire cortex as in maze-learning, or within a limited sensory area as in the retention of habits based upon the discrimination of light intensities. Are such non-focal symptoms due directly to the reduction in quantity of cerebral tissue or to pathological changes in parts of the cortex not directly involved in the lesion?

Lashley (1929) has shown that the deterioration in capacity for maze-learning persists for at least three months, which seems sufficient to eliminate shock or chemical disturbances from the presence of necrotic tissue. Of the factors which might produce a permanent general disturbance of cerebral function the most probable would seem to be circulatory disorders arising from mechanical distortion of the tissues or from a persistent hydrocephalus. The present experiments show conclusively that these factors (within the limits of their occurrence in experimental animals) have a negligible effect upon the formation of the maze habits. In the absence of any direct evidence for other causes of persistent metabolic disturbance

in the brains of operated animals, it seems safe to conclude that the non-focal symptoms are a direct result of the absence of the tissue destroyed.

SUMMARY

The effects of dilatation of the lateral and third ventricles of the brain upon the rat's ability to learn the maze have been tested. Artificial enlargement of the ventricles to as much as 60 times their normal volume by injection of oil is followed by only a slight retardation in learning, which is scarcely greater than would be expected from the damage to the cortex by the injecting needle. The records of 22 animals with spontaneous hydrocephalus are not significantly different from those of others having equal amounts of cortical destruction and no hydrocephalus. The evidence rules out trophic disturbances arising from mechanical distortion of tissue or increased intracranial pressure as a factor in producing correlation between extent of cerebral destruction and efficiency in learning.

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ELECTROMYOGRAPHIC STUDIES OF THE GASTRO-INTESTINAL TRACT

III. Observations on Excised Intestine

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In a previous publication, Berkson, Baldes and Alvarez have described experiments which show that during rhythmic contraction of the intact small intestine, rhythmic variations in electrical potential occur which, when recorded, have a characteristic wave form. The phases of this wave bear a definite relation to the phases of the mechanical movement of contraction and relaxation. Briefly, the relationship may be described as follows: The potential wave has a symmetrical form, the first half of which corresponds to contraction, the second to relaxation; the contraction phase of the potential wave contains a rapidly oscillating part, not present in the relaxation phase.

Since the vagal and sympathetic nerves were intact in the experiments reported, the question was open as to whether the origin of the potential variations observed was in the extrinsic nervous system or was situated in the structure of the intestine itself.

It has since been found that the same form of potential wave is present in excised intestine. Pieces about 3 cm. long were excised from various parts of the small intestine of rabbits and allowed to contract rhythmically in Ringer-Locke's solution kept at 37°C. The solution was contained in a glass vessel of about 100 cc. capacity; one electrode (silver-silver chloride) was kept in the bath, the other was attached to the piece of intestine, and a layer of mineral oil was placed on the surface of the bath to envelop the intestine. Simultaneous electrical and mechanical records were made on moving bromide paper by means of an apparatus previously described (1). Except where specifically stated otherwise, unipolar records were studied.

In figure 1 is shown an example of a simultaneous electrical and mechanical record so obtained. It is seen that all the distinctive features of the potential wave described for intact intestine are present here, in the same

[1] Work done on a grant from the Josiah Macy Jr. Foundation, under direction of Walter C. Alvarez, Associate Professor of Medicine, The Mayo Foundation, University of Minnesota.

relationship to the mechanical movement of contraction and relaxation as established for intact intestine. It was concluded, therefore, that this wave must have its origin within the structure of the intestine and not in the extrinsic nervous system.

Similarly it has been possible to obtain the same form of wave from the small intestine of the white rat, and also of the guinea pig. An attempt has been made to repeat the experiment with excised intestine of the dog and of the cat, but it is difficult to record regular rhythmic contractions from short, excised pieces of intestine of these animals. However, electrodes have been inserted operatively, under aseptic conditions, into the small intestine of the dog and of the cat, and after about a week has been

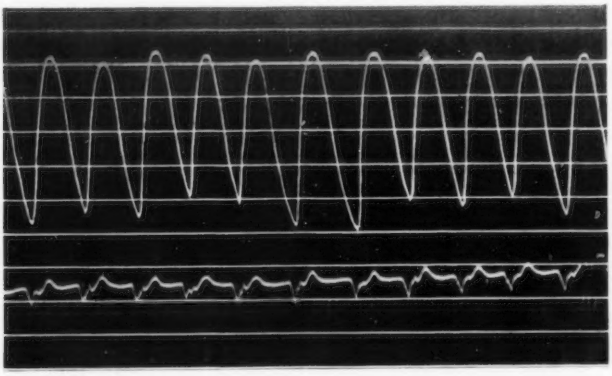

Fig. 1. A simultaneous mechanical and electrical record from excised intestine of rabbit. The mechanical is above the electrical record. One electrode was in the bath, the other on the intestine. Upward movement on the mechanogram indicates contraction; on the electrogram, upward deflection indicates increasing negativity of the tissue relative to the bath. The time interval is five seconds.

allowed for healing records have been obtained that showed the same form of potential wave. Taking all this experience together, namely, that in the rabbit, rat, guinea pig, dog, and cat the same distinctive wave of potential variation is seen, it may be concluded that it reflects some basic element in the physiology of the small intestine.

A number of exploratory experiments were performed to determine the effect on the electrogram of various external forces applied to the excised intestine. 1. Electrical shocks from an inductorium were thrown into rhythmically contracting intestine, while an electromyographic record was being taken. Each shock produced a marked contraction, and at the time of stimulation there was a quick throw of the galvanometer string usually in the positive direction. The latter was due probably to stimulus

escape. Immediately afterward there was resumption of the regular rhythmic form of wave in regular relation to the mechanical movement. No sustained or marked effect on the electrical wave was noted (fig. 2*a*). 2. If the same experiment is performed with intestine that has ceased contracting rhythmically, at each stimulation there is again a marked but slow

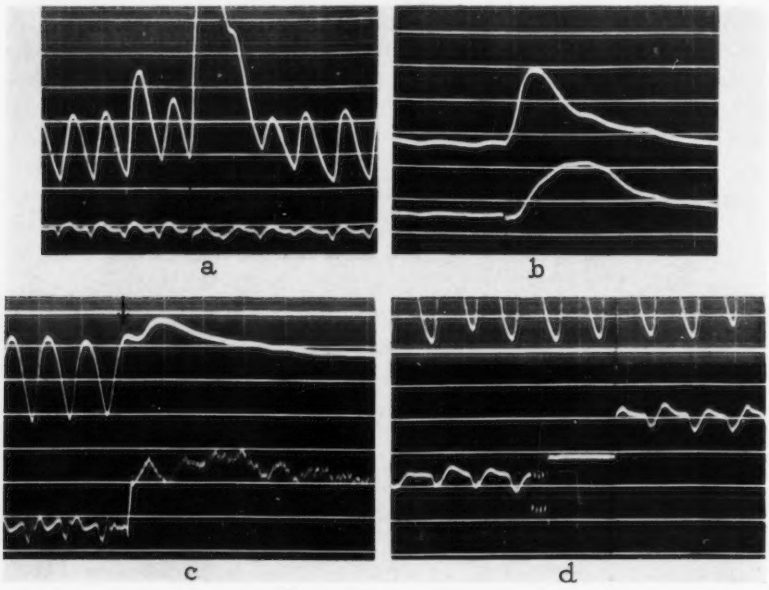

Fig. 2*a*. Electrical stimulation of rhythmically contracting intestine. Above, mechanical record; below, electrical record. There is a short positive deflection at the instant of stimulation, probably stimulus escape. No marked effect on the electrical record is noted; *b*, the same in an intestine which has ceased contracting rhythmically. Stimulus produced a contraction and a marked negative deflection; *c*, tetanus produced by addition of barium chloride to the bath. The galvanometer string executes rapid oscillations which resemble those seen briefly during the contraction phases of the normal wave; *d*, to test whether the galvanometer deflections of the characteristic wave are due to resistance changes or potential variation. Left, string adjusted to positive side of zero position (shown as straight line). On the right, continuous record, with string adjusted to negative side of zero. The wave should be inverted on both sides of zero if it has its origin in resistance changes of the tissue.

contraction. However, with each contraction there is now a deflection of the string in the negative direction sustained during the entire period of contraction, and a slow return during relaxation (fig. 2*b*). 3. Stretching of the intestine and shortening it passively by pushing the movable attachment of the enterograph toward the fixed attachment, produces inconstant

effects. Sometimes the result is clear-cut; stretching produces a positive variation, shortening a negative deflection. But in a number of experiments no effect was visible. 4. If the intestine is thrown into tetanic contraction, the galvanometer string executes a rapid oscillation at the rate of about 5 each second. Figure 2 *c* shows the record of such an experiment in which tetanus was produced by the addition of barium chloride to the bath. These oscillations have the appearance of what is seen briefly during the contraction phase of the normal rhythmic electrical wave, and it is suggested that during normal rhythm they correspond to a tetanic like state incident to contraction.

The question has been raised as to whether the regular wave forms that have been described are due to changes of resistance of the intestine as it contracts and relaxes, rather than to variations of potential. It is suggested that if, in the galvanometer circuit, any existing polarization voltage is not completely compensated by the voltage of the potentiometer circuit, a change in resistance of the intestine will produce a deflection of the galvanometer string. As the intestine contracted and relaxed, deflections would occur that might easily appear as the wave we have described.

A crucial test of this hypothesis can be made on the basis of the following considerations. Suppose a net voltage of $-v$ exists in the circuit; when the intestine contracts the string moves in the negative direction. If the deflection is due to a change of resistance it must have been a decrease, since the galvanometer deflection was in the direction of the voltage. If the compensating circuit is adjusted so that the net voltage in the galvanometer circuit is positive, a deflection produced by contraction of the muscle now, if it is due to a change of resistance, will be in the positive direction, that is, opposite to the first experience. On the other hand, if it is produced by a change of potential related to the contractual process, the deflection will be in the original, that is, negative direction. From these observations it is clear that the wave form observed, if it is due to changes of resistance of the intestine, will be inverted when the net voltage in the circuit is changed from a negative to a positive value. In figure 2*d* is shown a record made of a wave, first with the string adjusted to the positive side of zero line, and continuous with this the same record with the string adjusted to the negative side of the zero position. It is seen that the wave maintains the same orientation at both adjustments. This constitutes a sufficient proof that the deflections of the string recorded in the characteristic wave are due to variations of potential at the site of the electrode attachment to the intestine.

SUMMARY

A characteristic wave of potential variation, previously observed during rhythmic contraction of the intact intestine, is now found in experiments

with excised intestine. This is taken to show that the origin of the wave is in the structure of the intestine, and not in the extrinsic nervous system. It is proved that the observed wave must originate in potential variations at the site of the electrode attachment to the intestine and not in changes of electrical resistance of the tissue. Experiments are presented in which the electrical changes are observed while the intestine is exposed to a variety of external forces. It is found that tetanus produces rapid oscillation of the galvanometer string similar to that observed during the contraction phase of the normal wave.

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ELECTROMYOGRAPHIC STUDIES OF THE GASTRO-INTESTINAL TRACT

IV. An Inquiry into the Origin of the Potential Variations of Rhythmic Contraction in the Intestine; Evidence in Disfavor of Muscle Action Currents

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The concept of action current implies a necessary association between action and current. According to prevailing ideas, the electrical phenomenon is a manifestation of the excitation that precedes physiologic activity. When one occurs the other must be present, although either may be difficult to observe. To be sure, a number of experimenters have shown that the contraction of the heart can be arrested by various means which leave the electrogram relatively unaffected (2), (5), (6), and who claim therefore that the current can exist without muscular contraction. But the authority is equally weighty on the other side, which claims that these observations are accounted for by lack of sensitivity of the recording apparatus, and that a sufficiently delicate instrument always shows mechanical activity when there is an electrical wave (4).

In the case of the electrical manifestations of intestinal activity which have been reported in this series, there are a number of considerations which weigh against the probability that what have been observed are muscle action currents. Some of these were considered in the first paper. They are chiefly concerned with the relationship of the electrical wave to the phases of the mechanical contraction and relaxation. Whereas the true action current of muscle is terminated before completion of contraction, and there is no current during relaxation, it is found that, in the intestines, the potential variation proceeds pari passu with the mechanical contraction and relaxation. For every point in the mechanical movement, there is a corresponding point in the potential wave, and this is true during relaxation, when no action current at all would be expected.

Still further question as to the identity of the observed potential variations with muscular action currents, as usually conceived, is prompted by

[1] Work done on a grant from the Josiah Macy, Jr. Foundation under the direction of Walter C. Alvarez, Associate Professor of Medicine, The Mayo Foundation, University of Minnesota.

the lack of constancy of the occurrence of the mechanical activity with the electrical manifestation. As has been reported previously, in the intact intestine there often is found a very clear and definite relationship between electrical and mechanical activity, but it is not at all unusual for erratic interruptions to occur in this correlation. The recorded electrical waves may completely disappear, while the mechanical activity continues unabated, or vice versa. When these events are observed in the intact intestine, it is easy to attribute them to the multifarious activities of various parts of the gastro-intestinal tract with resulting mutual interference of

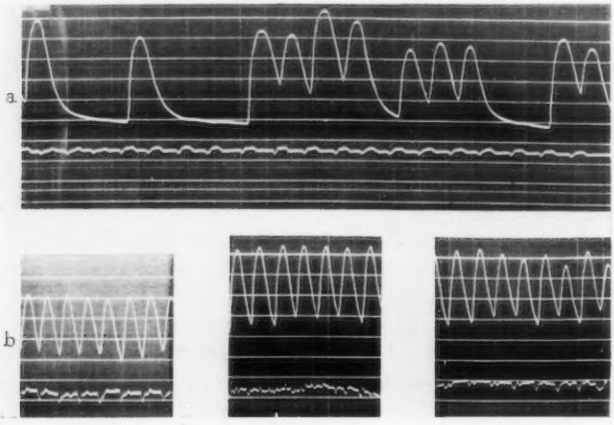

Fig. 1 *a*. Spontaneous dissociation of mechanical and electrical rhythm. The mechanical movement (upper record) ceases periodically during several cycles, while the electrical (lower record) continues; *b*, an example of erratic oscillations of the galvanometer string during the course of a regular record, which are not reflected in any change of the recorded mechanical movement. The illustrations are from a long continuous record. Left, five minutes after excision of the intestine. Middle, two minutes later; right, two minutes after middle. The regular record continued for a long time, interrupted periodically by oscillations like those illustrated in the middle picture.

the different parts. But in excised intestine an explanation of this sort is inadmissible.

In excised intestinal strips, observed under conditions described in the preceding paper, is encountered a form of spontaneous dissociation between electrical and mechanical activity that appears to be characteristic of certain conditions of the intestine. The mechanical rhythm will, for a considerable time, be in perfect correlation with the electrical variations, when, for reasons at present unaccountable, the mechanical movement will cease for short periods while the electrical variations proceed without any observ-

able modification. Following this, the mechanical rhythm appears again in perfect synchronism with the electrical waves. An example of this may be noted in figure 1*a*. Again, there is commonly seen during an experimental "run," transient, rapid, oscillations of the string, that appear to affect the mechanical movement not at all (fig. 1*b*).

More convincing than these passing observations is the fact that such dissociation can be produced experimentally by application of drugs. Here are reported two definitive experiments: 1, the complete arrest of recorded mechanical movement with continuation of the electrical changes, by means of epinephrine, and 2, the disappearance of the potential wave, while the mechanical rhythm is in full progress, when nicotine is applied.

The first experiment was performed on the intact intestine of a rabbit placed in a bath of saline solution, the intestines enveloped in mineral oil, and three pairs of electrode recorders adjusted as described in the initial paper of this series. The galvanometer was attached to the most aboral pair of the three, and a bipolar electrical record was made simultaneously with the mechanical record. Fifteen-hundredths cubic centimeter of epinephrine 1:1000 was injected into the ear vein. Immediately, at all recorders, there was cessation of the recorded mechanical movement. The electrical waves which were being recorded from the third electrode remained unaffected, except that the frequency of the waves increased (fig. 2). The same effect of arrest of the mechanical movement without any disturbance of the electrical wave, by means of epinephrine, can easily be demonstrated in excised intestine. Alvarez, in 1922, noted that epinephrine abolished the mechanical activity but affected only slightly the concomitant electrical variation. Puestow, working with intestine of dog transplanted to the abdominal wall, observed the same phenomenon and so also has Castleton in unpublished experiments from this laboratory.

In the second experiment, a piece of intestine 5 cm. long was excised from the rabbit and allowed to contract rhythmically in a small glass vessel containing 80 cc. of Ringer-Locke's solution, maintained at 37°C. The surface of the bath was covered with a layer of mineral oil, and simultaneous electrical and mechanical records were made. The records showed clearly defined waves of potential variation in characteristic relation to the phases of the mechanical movement of the muscle. Half a cubic centimeter of nicotine diluted 1:100 was added to the bath. Immediately, the mechanical and electrical records gave evidence of marked disturbances. The mechanogram exhibited a vigorous, sustained contraction, and the electrogram portrayed violent erratic changes. This continued for about four minutes, during which time the electrical record showed a series of very small regular waves interrupted by erratic variations. Curiously, many of the waves which appeared were small, regular forms, miniatures of the waves seen during normal rhythmic contraction. The experiment was

allowed to continue five hours and observations were made periodically during this time. The progress of the experiment may be noted in figure 3. At the end of five minutes after application of nicotine, regular rhythmic movements of the muscle began to return; the electrical record, however, gave evidence of only feeble, periodic pulsations, quite different from the normal waves. At the end of fourteen minutes the mechanical record gave evidence of regular, vigorous contractions of large amplitude and slow rate (6 per minute). The electrical changes recorded at the same time were practically nil, only hardly perceptible pulsations appearing. At

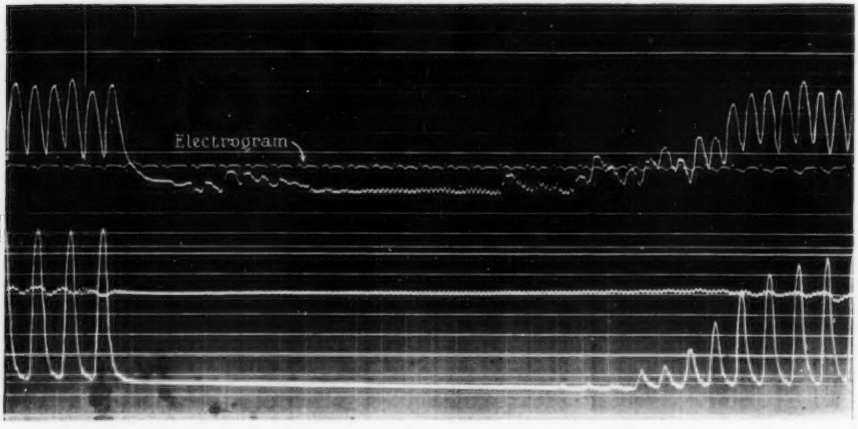

Fig. 2. A record made from an intact intestine of a rabbit, to show the effect of epinephrine. From above downward, the first, third, and fourth are mechanical records; in the middle one of these the attachment was across the intestine to get the movement of the circular fibers, the other two were longitudinal attachments. The galvanometer attachment to the lowest recorder was bipolar. The electrogram is immediately below the uppermost myogram. The potential wave is seen to be inverted (3) and continues after epinephrine (0.15 cc. 1:1000) injected into ear vein had stopped the recorded mechanical movement.

the end of seventy-seven minutes the mechanical movement was continuing in regular, vigorous rhythm, but no electrical changes were visible. At three hours the mechanical movement began to be feeble and irregular, with no movement of the galvanometer string, and at the end of five hours both were quiescent.

Such experiments with epinephrine and nicotine have been repeated many times with uniform results, as described. Moreover, these two are not the only drugs which effect a dissociation of the mechanical and electrical manifestations of rhythmic contraction. Cyanides, cocaine, pilocarpine,

physostigmine, as well as other agents affect differentially, but in a less striking degree than epinephrine and nicotine, the two aspects of rhythmic activity. These latter experiments are to be reported in detail at a later date.

From the experiments reviewed, it appears virtually established that the electrical phenomena which have been reported in this series are, in some important way, to be distinguished from muscle action currents. To be sure, they do, under ordinary conditions accompany muscular activity in a regular way, and, as has been demonstrated in the preceding paper, they have their origin in potential variations in the intestine. In so far, they

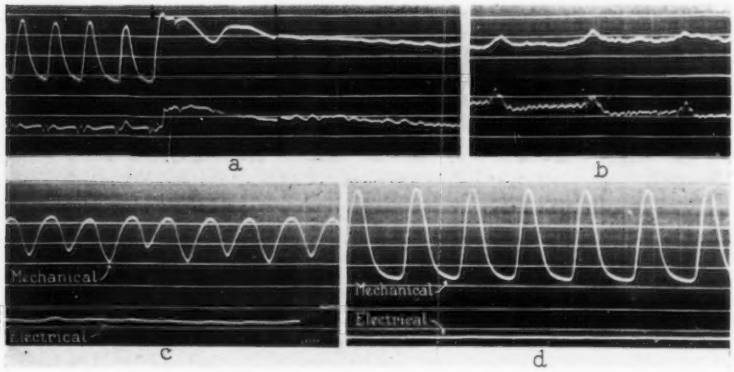

Fig. 3. The effect of nicotine; excised intestine; *a*, normal rhythm. At arrow, 0.5 cc. 1:100 nicotine added to bath; immediately a sustained contraction shown in the mechanical record (above) and erratic oscillations in electrical (below); *b*, two minutes later, small potential waves of characteristic form; *c*, seven minutes after application of nicotine. The mechanical movement is returning. The electrogram gives evidence of only feeble pulsations; *d*, one hour after nicotine, the mechanical movement is vigorous and regular, no concomitant electrical changes are visible.

are akin to muscle action currents. But the relation of the potential variations to the phases of the contractual rhythm is fundamentally different from that which characterizes muscle action currents, and the fact that the potential variations may be dissociated from the mechanical movement of the muscle distinguishes them completely.

There is the possibility that the electrical variations have their origin in the intrinsic nervous structure of the intestine. The fact that nicotine, which has been proved to paralyze the synapses of sympathetic nerves, can abolish the recorded electrical variations, would tend to corroborate this hypothesis. But it must be remembered that nicotine affects also muscle tissue, so that this evidence is by no means conclusive. However, since, as

has been demonstrated, the electrical waves may proceed in perfectly normal, regular rhythm, when there is no perceptible muscular activity, it is difficult to account for them except on a nervous basis. On this basis, a working hypothesis may be framed somewhat as follows: There occur in the intestine regular rhythmic impulses which emanate from the intrinsic nervous plexus. These serve as a regulating force for the muscular rhythm, but do not originate it. When they are abolished by drugs or other means, the muscular rhythm, essentially myogenic in origin, proceeds autonomously. It is notable that when, as after nicotine, the electrical variations have disappeared, but the muscular rhythm remains, that rhythm is more even, and of much slower rate (6 per minute) than is normally seen when an electrical wave is evident. If this hypothesis is essentially sound, it must further be supposed that the forces that produce the potential waves have some important rôle in regulating the normal movements of the intestine. In this line of thought further experiments are planned.

SUMMARY

The relationship of the phases of the potential variations observed during rhythmic contraction of the small intestine to the phases of mechanical movement militates against the view that the potential changes originate as muscle action currents. Furthermore dissociation of the electrical and mechanical activity occurs spontaneously, and can be effected experimentally by means of drugs. The hypothesis is put forward that the rhythmic electrical changes reflect rhythmic pulsations that emanate from the intrinsic nervous plexus of the intestine, and that these have a regulating function in the physiology of the small bowel.

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THE INFLUENCE OF THE VAGUS NERVE ON ESOPHAGEAL SECRETION

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The stomach, pancreas and small intestine are known to receive secretory fibres through the vagi nerves. In the larynx and trachea, which are embryologically derived from the primitive tubular gut, there are also glands innervated by the vagus (Kokin, 1896, recently confirmed by Florey, Carleton and Wells, 1932). Hence it may be supposed that the glands of the esophageal mucosa are innervated by the vagi nerves. Although it is known histologically that these glands are of two kinds—esophageal and cardiac esophageal glands, nothing is known regarding their innervation. During the course of a previous investigation by one of us (A.M.V., 1931) it was necessary to isolate the esophagus of a dog from the pharynx above and the stomach below. When the vagi nerves were subsequently stimulated there was a definite flow of mucoid-like material from the esophageal cannulae. This we believe to be a secretion from the esophageal glands. In the present investigation we have studied this esophageal secretion obtained following stimulation of the vagi nerves, paying particular attention to the course of the secretion and its chemical properties.

METHODS. The specific gravity of the secretion was determined by picnometer and the pH by the indicator method of Clark (1928) and Lubs, the samples not having been collected under oil. The acid-combining power was determined by heating 1 cc. of the secretion with 5 cc. of n/50 H_2SO_4 in a boiling-water bath for five minutes and by back titration with n/50 NaOH, using methyl-red as an indicator; it is expressed in milli-equivalents per litre. Nitrogen was determined by Pregl's (1930) micro-Kjeldahl procedure. The nitrogen content of the filtrates obtained on precipitation of the mucus secreted with four volumes of absolute alcohol is interpreted as non-protein nitrogen. Amino-nitrogen was determined by formol titration according to the method of Henriques and Sörensen (1910), urea and ammonia by the method of Van Slyke and Cullen (1914—see Peters and Van Slyke, 1932, p. 547). Potassium was determined by the method of Kramer and Tisdall (1921), calcium by the method of Clark and Collip (1925). Chlorine was determined by the method of Wilson and Ball (1928), phosphorus (total) by the method of Fiske and Subbarow (1925),

the wet procedure in which sulphuric and nitric acids are employed being used for digestion. Pepsin was determined by the Mett's tube procedure as modified by Nirenstein and Schiff, with an incubation period of from one to three days.

EXPERIMENTAL RESULTS. Dogs were used in the experiments, altogether eight experiments being completed. The esophageal secretion obtained was chemically analysed in each case. In the two following experiments a more detailed examination of its composition was made. The results of these two experiments are presented in table 1 and the course of the secretion in figure 1. The vagi nerves were rhythmically and continuously stimulated in the neck for long periods (nine and twelve hours respectively). A very thick mucus-like substance was collected from the upper and lower esophageal fistulae. In all the experiments more secretion was obtained from the upper esophageal fistula than from the lower one e.g., in experiments I and II, 24.8 and 35.0 cc. respectively were obtained from the upper cannula and only 9.3 and 3.5 cc. from the lower cannula.

TABLE 1

Composition of the esophageal secretion in two dogs (expts. of April 9 and April 23 respectively), collected from the upper and lower ends of the esophagus during rhythmic stimulation of the vagi in the neck

In each experiment sample I and sample II correspond to the secretion collected during the first and second periods of stimulation.

SECRETION	EXPERIMENT, APRIL 9			EXPERIMENT, APRIL 23	
	Sample I (upper end)	Sample II (upper end)	Sample III (lower end)	Sample I (upper end)	Sample II (upper end)
Time of collection (min.)	315	375	690	410	135
Quantity (cc.)	5.9	18.9	9.3	17.0	18.0
Specific gravity (Density)		1007.5			1007.0
pH		8.3		7.5	8.0
Acid-combining power (m.eq./1 litre)		15.0	15.0	11.5	11.6
Solids (gm. %)		1.353		1.477	1.25
Ash (gm. %)		1.05		0.957	0.99
Organic material (gm. %)		0.303		0.52	0.26
Cl (mgm. %)		618.0	501.0	504.7	582.8
P—total (mgm. %)		1.2		1.2	0.9
K (mgm. %)		53.6		51.0	58.2
Ca (mgm. %)		6.2		6.9	6.9
Nitrogen—total (mgm. %)	142.4	63.3	85.1	80.6	52.5
Nitrogen—non-protein (mgm. %)		39.2		36.0	31.6
Nitrogen—amino (formol) (mgm. %)		2.6		2.5	2.5
Nitrogen—ammonia (mgm. %)	4.1	3.64	4.48	4.76	4.72
Nitrogen—urea (mgm. %)		6.72	5.88	5.04	4.78
Peptic power	0	0	0	0	0

The vagi were stimulated alternately with an induction current, commencing with a current of 9 cm. and in some experiments increasing it to 6 cm.

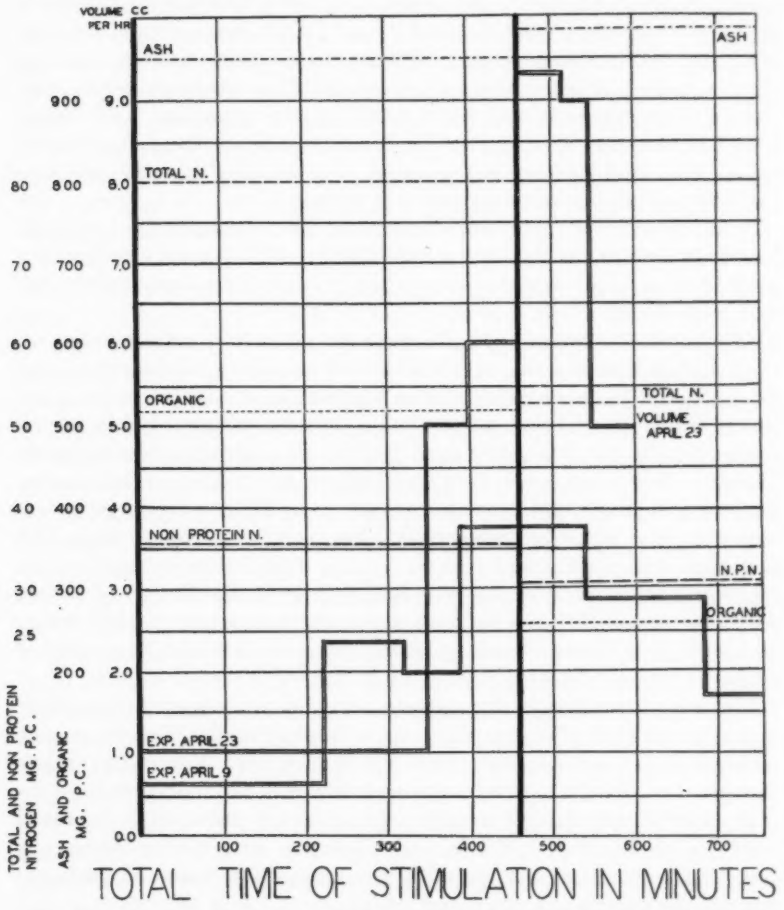

Fig. 1. The course of the esophageal secretion (continuous lines) in the experiments of April 9 and April 23 is shown on the chart. In addition in the experiment of April 23 the concentration of different substances in the mucus during the two periods of secretion is demonstrated (the chart is divided by the heavy line into two parts, representing the two periods of secretion).

A strong current was used because we have found in previous work that weak induction currents which activated a flow of mucoid fluid from the stomach failed to stimulate esophageal secretion. In control experiments

when the vagi were not stimulated there was scarcely any esophageal secretion. From figure 1 it is evident that there is a long initial period of secretion lasting for 200 to 300 minutes. During this period the rate of secretion was 0.55 cc. (expt. of April 9) and 1.0 cc. (expt. of April 23) from the upper end of the esophagus. The secretion during this stage consists of an extremely thick and tenacious mucoid-like substance, which precedes a more copious flow of semi-fluid mucoid-like substance. We do not consider this a true latent period but believe it is due to the difficulty with which this viscid material is evacuated. On standing at room temperature this material turned to a jelly-like mass.

Secretion was well maintained for the next 200 to 300 minutes, gradually falling off toward the end of the experiment. This terminal decline was probably due to diminished nerve excitability and to the fact that the animals were dying.

The secretion collected from the upper end of the esophagus during each of these experiments (table 1) was separated into two parts (samples I and II). In addition in the experiment of April 9 the secretion from the lower end of the esophagus was collected as a separate sample It will be observed in table 1 that the reaction of the esophageal secretion was decidedly alkaline. It was rich in ash with a moderate content of organic substances. The initial secretion was extremely thick, but as the flow increased in volume it became somewhat more fluid. The secretion obtained during the first part of the experiment (sample I, expt. April 23) contained twice as much organic material as that collected during the second part of the experiment (sample II). Since the character of the secretion changed during its course from highly viscous to fluid, it is probable that the sources of the mucus were exhausted during the prolonged stimulation.

The total nitrogen in both experiments was definitely lower in samples of secretion obtained after secretion was well established. The non-protein nitrogen and urea, however, were less diminished. Values for amino-nitrogen and ammonia were unchanged, whereas the inorganic constituents varied during the two periods. The total ash increased in sample II (expt. April 23), both chlorine and potassium rose during the second stage of the secretion, and the calcium concentration remained unaltered whereas the phosphorus concentration fell.

All these data indicate that the stimulation of the vagi activated a true secretion from the esophageal mucosa. If the process were merely one of squeezing out already formed mucus, the secretion would not continue so long nor would its chemical composition be changed. These chemical changes are similar to those which are known to occur in the composition of saliva during prolonged activity of the mixed glands.

The composition of the secretion collected from the lower end of the

esophagus differed but slightly from that obtained from its upper end (sample III, expt. April 9).

No definite results were obtained in the experiments involving atropinisation of the animals during the course of the esophageal secretion. Although the vagus innervation to the heart and other organs was quite paralysed by doses of 10 to 15 mgm. atropine sulphate, mucus continued to flow slowly from the upper cannula. This was probably due to the fact, as shown by post-mortem examination, that a great amount of viscous material still adhered to the walls of the esophagus. This secretion is gradually evacuated from the organ by the respiratory movements and probably also by the contraction of the esophagus.

A comparison of the chemical properties of the esophageal, salivary and gastric glands is of interest. It must be remembered, however, that the chemical composition of the secretion varies with the rate of secretion and the strength of the stimulus. Nevertheless we feel that certain general principles can be established. (The data here presented on the inorganic composition of saliva were obtained by reflex stimulation, being some unpublished results of Dr. H. Baxter.) The acid-combining power of the esophageal secretion is lower than that of saliva from the parotid gland (25.0 to 65.0 mil. equiv.) and lower than that of saliva from the mixed glands (about 15.0 to 30.0 mil. equiv.). In total ash content the esophageal secretion is much richer (about 1.0 per cent) than the secretion of the mixed glands (0.29 to 0.611 per cent) or the parotid glands (about 0.54 to 0.89 per cent). The ash content of the gastric juice according to Rosemann (1907) is still lower (0.127 to 0.136 per cent). The same is true regarding the solids of dog's gastric juice, viz., 0.315 to 0.88 per cent according to Kersten (1920) and 0.387 per cent according to Rosemann (1907). The chlorine concentration in the esophageal secretion (500.0 to 618.0 mgm. per cent) is about equal to or somewhat lower than that of the gastric juice (about 0.6 per cent (Rosemann, 1907); 0.587 to 629 per cent (Webster, 1931)) and much higher than in the saliva of the mixed glands (58.0 to 246.0 mgm. per cent) and parotid glands (170.0 to 328.0 mgm. per cent). Potassium is lower in the esophageal secretion (51.0 to 58.2 mgm. per cent) than in the saliva of the mixed glands (48.0 to 92.5 mgm. per cent) and parotid glands (37.9 to 91.6 mgm. per cent), but higher than in the gastric juice (30.7 to 43.3 mgm. per cent (Rosemann, 1907)).

The calcium concentration in the esophageal secretion (6.2 to 6.9 mgm. per cent) is higher than in the gastric juice (0.22 to 0.07 mgm. per cent) but lower than in the saliva of the mixed glands (5.8 to 13.2 mgm. per cent) or of the parotid glands (20.0 to 26.0 mgm. per cent). A large proportion of the total nitrogen present is in the form of non-protein nitrogen. The same seems to be true of the nitrogen in the gastric juice. During the

course of secretion there is a marked fall in the total nitrogen with only slight diminution of the non-protein nitrogen. The concentration of ammonia (4.72 to 4.76 mgm. per cent) in the esophageal secretion is almost the same as in the gastric juice, whereas the concentration of urea is higher in the former (4.78 to 6.72 mgm. per cent) than in the latter (0.16 to 0.88 mgm. per cent). No pepsin was demonstrated in the esophageal secretion.

Because of the chemical differences cited above, it seems justifiable to conclude that the esophageal secretion is a special secretion which differs from the secretions of the adjacent upper and lower parts of the alimentary canal.

Discussion. In order to interpret physiological findings it is important first to consider the histological structure of the organ under observation. Thus in the case of the esophagus we find that in the dog there are two kinds of esophageal glands—1, esophageal glands, and 2, cardiac esophageal glands. The "esophageal glands" are numerous in the esophagus of the dog and are composed of two kinds of cells—mucous and demilune (Klein, 1879; Renant, 1897, Helm, 1907; Goetsch, 1910; Ellenberger, 1911). Goetsch reports an arrangement of the serous complexes which resembles that found in the submaxillary gland. These are provided with intercellular secretory capillaries. Situated in the main and sometimes in the secondary excretory ducts are ampullae filled with mucoid secretion (Schaffer, 1897; Goetsch, 1910). Of the second type of gland found in the esophagus, viz., the "upper and lower cardiac esophageal glands," two different kinds have been described. Some have the same structure as the glands of the cardia and are practically free from parietal cells, while others, which form what might be called "islands of gastric mucosa," are glands of a true fundic type and secrete acid gastric juice (Schaffer, 1904; Schridde, 1904, 1905, 1908; Helm, 1907; Ellenberger, 1911). The upper cardiac esophageal glands are absent in the dog, whereas the lower cardiac esophageal glands have been found in the dog alone of all the domestic animals, and then only occasionally (Helm, 1907; Ellenberger, 1911).

Thus from the histology of the esophagus we learn that the glands of this organ are of mixed type, hence their secretion is of a compound nature. During the first period of stimulation of the vagus the secretion is very viscid and mucoid-like. The mucus of the esophageal secretion is secreted chiefly by the mucous cells of the esophageal glands and is partly derived from the ampullae of the excretory duct, where it is accumulated during the period of rest. The secretion of the demilune cells in this period is over-shadowed by the secretion of the mucous cells. After prolonged stimulation of the secretory nerve these sources of mucus were more or less exhausted and the secretion acquired a more fluid consistency. This explains also the fall in the concentration of total nitrogen and the rise in

the concentration of chloride and potassium in the secretion collected during the later hours of stimulation.

There was no demonstrable peptic activity in the secretion obtained from either the upper or lower end of the esophagus. This may be explained by the fact that the cardiac glands are rarely found in the esophagus of a dog. There is thus a close relationship between the esophageal secretion and stimulation of the vagi. In addition there are typical changes in the character of the secretion during stimulation. We thus conclude that the vagus is a secretory nerve to the esophageal mucosa. The part played by the vagus in the esophageal secretion is important experimentally whenever the influence of this nerve on the gastric secretion is studied. It is evident that the possibility of admixture of the esophageal secretion with the latter must be excluded. The data of Kokin (1896) and Florey, Carleton and Wells (1932) concerning the innervation by the vagus of the glands of the larynx and trachea are in agreement with our findings. It is to be expected that the innervation of the larynx and trachea should be similar to that of the esophagus, since the respiratory ducts are developed as a ventro-medial outgrowth from the part of the fore-gut which later develops into the esophagus.

From the phylogenetic point of view according to Goetsch (1910) the presence of esophageal glands in some animals (chiefly mixed feeders) and their absence in others (purely vegetable feeders) make it difficult to understand their rôle. He believes that the glands developed independently in different species in accordance with their special needs. In the dog large pieces of meat or bones require lubrication during their passage through the esophagus. It seems significant that the same nerve which regulates the motility of the esophagus can at the same time stimulate the secretion of the esophageal glands.

SUMMARY

1. Rhythmic stimulation of the vagi in the neck activates a secretion of mucoid fluid emanating from both the upper and lower ends of the esophagus in the dog.

2. The secretion obtained during the first period of stimulation of the nerves is extremely thick and tenacious. In the second period of stimulation there is a more copious flow of semi-fluid mucoid-like material.

3. The chemical composition of the esophageal secretion and its changes during prolonged stimulation were studied. These changes indicate that the action of the vagus produces a true secretion from the esophageal glands.

This investigation was carried out under the direction of Dr. B. P. Babkin, whose criticism and advice the authors gratefully acknowledge.

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THE FREQUENCY OF IMPULSES IN THE NERVE FIBERS TO THE NICTITATING MEMBRANE OF THE CAT

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In 1914 Cannon suggested that the outlying neurones of the sympathetic nervous system might transform the frequency of nervous impulses reaching them from a central source and thus adapt them to the peculiarities of the tissues innervated. To test this hypothesis Querido (1924) studied the effects of stimulating at various frequencies the preganglionic and postganglionic fibers to the nictitating membrane of the cat. As a result of an ingenious series of experiments he concluded that the optimal frequency for stimulation of the postganglionic fibers lay between 120 and 160 per second and that impulses arriving over the preganglionic fibers at either higher or lower rates were transformed in the superior cervical ganglion to this optimal frequency.

Adrian and Bronk in 1928 succeeded in recording the nerve impulses in single fibers of the phrenic nerve and found that the discharges associated with respiration showed frequencies of between 20 and 100 per second. This immediately threw grave doubt on Querido's conclusions, since all of the properties of sympathetic fibers indicate a slower rate of activity in them than in medullated fibers. Veach and Pereira (1925) had already criticized Querido's conclusion as to the transformation of the frequency of impulses in the superior cervical ganglion, but they did not question the order of magnitude of Querido's figures. We therefore undertook to repeat Querido's experiments with more rigid precautions.

Since the beginning of our experiments the work of Adrian, Bronk and Phillips (1932), Bishop and Heinbecker (1932), Rosenblueth (1932) and Orias (1932) has left little doubt but that the frequency of impulses in the nerves to the nictitating membrane is relatively low, probably not over 30 or 40 per second, and our own results are entirely in accord with this conclusion. We believe that we have in addition discovered certain important sources of error in the earlier experiments of Querido and of Veach and Pereira.

METHOD. For stimulation we first employed the rotary interrupter used by Querido and a balanced inductorium similar to the one described by Bishop (1927). Cathode ray oscillograph records obtained by Doctor Bishop (personal communication) with this coil show that the make and

break shocks are not grossly different in intensity or duration if the iron cores are in place in the secondary coils and the resistance in the secondary circuit is approximately equal to that of our nerves (about 1000ω). Physiological tests of the effectiveness of make and break shocks on the frog's sciatic-gastrocnemius preparation and also on the cat's soleus nerve-muscle preparation likewise show that under these conditions makes and breaks are very nearly equivalent. We therefore count both make and break shocks as stimuli instead of disregarding the makes as Querido and also Veach and Pereira did. We soon found, however, that a neon tube stimulating circuit closely resembling that of Briscoe and Leyshon (1929) was more satisfactory. In addition to eliminating all uncertainty as to the effective frequency, it is more regular in its operation. Such a circuit, frequently calibrated by galvanometric records, was employed in most of our significant experiments.

The surgical approach to the superior cervical ganglia and the methods of recording the contractions of the nictitating membrane isotonically on a smoked drum were identical with those employed by Querido (1924). Urethane anesthesia was employed throughout. For the experiments in which preganglionic stimulation alone was employed the nerve was placed in tubular electrodes (Forbes, Davis and Lambert, 1930) and buried in the tissues. It was found advantageous, in experiments in which high strength of stimulation was required, to dissect the sympathetic nerve free from the vagus bundle and from connective tissue as completely as possible. Tissue fluid was excluded by means of vaseline, all of these precautions serving to diminish short-circuiting of the shock by inactive tissue. The only disadvantage of this procedure is that the nerve does not maintain a constant threshold for as long a time as with the ordinary preparation. For stimulation of postganglionic fibers the nerve was usually freed completely from surrounding tissue and all collaterals cut, as the fibers for the nictitating membrane run, not in the branch to the internal carotid artery, as Querido thought, but with the chief bundle of fibers entering the skull directly (Zernik, 1928). The electrodes used were simply small hooks of silver wire, about 2 mm. apart, which were hung on the nerve, the latter being suspended by a silk thread.

Results. It appeared to us that neither Querido nor Veach and Pereira had made a sufficiently systematic study of the different variables involved which might affect the extent of contraction of the membrane. These variables include the tension on the membrane, the intensity of stimulation, the duration of stimulation and the frequency of stimulation. In order to bring out clearly the relationships of the last factor, we desired to select values for the other factors such that unintentional minor fluctuations would not modify the results.

A satisfactory load for the membrane under our conditions of isotonic

recording appeared to be about 2 or 3 grams, and, what is more important, no extensive or consistent variation of contraction with load over a wide range could be detected.

The intensity of stimulation was next investigated, both with inductorium and with neon tube circuit, using several frequencies of stimulation but all below 30 per second. This test showed clearly the superiority of the neon tube circuit, since much smoother curves relating the extent of contraction to intensity of stimulation were obtained with it than with the inductorium. In all cases stimulation was continued long enough to allow the contraction to reach a maximum. These maxima were measured and plotted against intensity. The curves so obtained rise rapidly from the base line, sometimes showing a moderate initial concavity, and reach a plateau at about four times the strength of the threshold stimulus.[1] Beyond this, up to at least 10 or 12 times the threshold stimulus, a constant response is always obtained, independent of strength of stimulation, although different for different frequencies. The same type of curve was obtained for postganglionic as for preganglionic stimulation. In all cases the threshold was higher for the postganglionic fibers, but the ratio of maximal to threshold stimulus was the same. We therefore always began our observations by a determination of the threshold of the nerve under examination, and thereafter employed a stimulus of at least 5 times this strength. When frequencies of stimulation above 30 per second are employed, the relations of the strength of stimulus necessary for maximal response become more complicated, as will appear below. It is worth nothing, however, that all workers in this field apparently agree that the threshold of stimulation for the postganglionic fibers is higher than that for preganglionic, and we particularly wish to point out that Querido apparently never used definitely maximal stimulation in the case of the postganglionic fibers.

Another variable is the duration of the stimulation. We shall not go into detail on this point, since our results merely confirm those of Rosenblueth (1932), but shall simply point out that since the rate of contraction varies with the frequency of stimulation, it is necessary, for a proper comparison of different frequencies, to wait in each case until a definite plateau has been reached. With frequencies of stimulation less than 28 per second the plateau has not been reached at the end of 12 seconds. At 46 per second it is reached at the end of 7 seconds, and at 176 per second it is reached within 4 seconds. Some of the apparent discrepancies between the conclusions of Querido and of Veach and Pereira are to be explained on this basis, since Veach and Pereira worked for the most part with periods of one second of stimulation, while Querido usually maintained stimulation continuously

[1] The values here given assume a linear relationship between the strength of shock and the reading on the scale of the potentiometer of the neon tube circuit. The assumption is approximately true but not exact.

and apparently attained definite plateaus. Another difficulty with using short periods of stimulation alternating with periods of rest is that the contractions obtained in this way vary in amplitude, depending on the condition of the membrane and the amount of previous activity. On the other hand, with long continued stimulation the plateau which is finally reached is independent of the previous resting level and is not appreciably affected by the amount of activity shortly preceding the test in question.

When the preganglionic fibers are stimulated at various frequencies the resulting plateau of contraction is greater with higher frequencies up to 25 to 30 per second (fig. 1). This again confirms Rosenblueth's observations. With a strength of stimulus 5 or 6 times threshold, an increase of frequency

Fig. 1

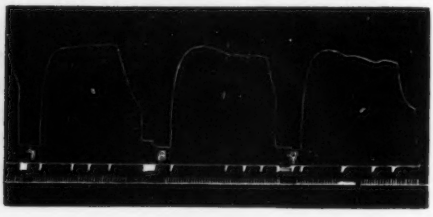

Fig. 2

Fig. 1. 5/6/31. Supramaximal stimulation of preganglionic fibers by neon tube stimulator at frequencies of 5, 10, 20 and 30 per second. Signals show changes of frequency and end of stimulation. Drum stopped during relaxation. Speed of drum as in figure 2.

Fig. 2. 5/4/31. Stimulation of preganglionic fibers at frequencies of 30, 50 and 70 per second. Signals during stimulation indicate increases in intensity of stimulation from 6 to 6.5 then to 7 times threshold. Contraction is well maintained at 30 per second. With frequencies of 50 and 70 there is a fall which is partially restored by a slight increase in the strength of stimulation. Note time scale in seconds. Same speed of drum in all experiments.

above 30 per second may result in a diminution in the contraction. If now the intensity of stimulation is increased somewhat, the original level is at least partially restored. The simplest interpretation of this phenomenon is that, at these higher frequencies, the nerve fibers do not respond to every stimulus until the strength of stimulation is increased (fig. 2). This would indicate that the *relatively* refractory period of the preganglionic fibers under these conditions of repetitive activity is at least 30 to 40 σ in duration, which is in reasonable agreement with Bishop and Heinbecker's value of 3.5 σ for the *absolutely* refractory period. Our value suggests also Bishop and Heinbecker's figure of 20 to 30 σ for the "refractory period of the ganglion cell," but the fact that we find the same relation of contraction to stimulation of the postganglionic fibers leads us to the interpretation

stated above. Examination of the stimulating shocks by means of a cathode ray oscillograph shows that under the conditions of our experiments there is no physical interference or "overlap" such as that invoked by Veach and Pereira to explain Querido's results. By sufficiently increasing the strength of stimulation a maximal contraction of the membrane can be obtained up to a frequency of at least 200 per second (fig. 3) but, as Bishop and Heinbecker point out, this does not prove that impulses are being set up at this frequency in the nerve. The fibers are presumably responding at some submultiple of this frequency or irregularly at a slower rate. Forbes and Rice (1929) showed that medullated nerve responds in this way to too rapid a frequency of stimulation.

In most of the experiments on postganglionic stimulation, precautions were taken to prevent unintentional stimulation of preganglionic fibers. Sometimes the nerve was ligated proximal to the ganglia and in some cases the ganglion was completely removed. In each case the nerve was removed at the end of the experiment and examined microscopically to confirm the point of ligation or the completeness of excision. In a few experiments the nerve and ganglion were left intact and stimulation of the preganglionic fibers and of postganglionic fibers was carried out alternately. The results with postganglionic stimulation were the same as in the experiments in which the ganglion had been completely removed.

The same relation between frequency of stimulation and the magnitude of contraction was observed for postganglionic as for preganglionic stimulation, the contraction being small for low frequencies and increasing up to 25 or 30 stimuli per second (fig. 4). On increasing the frequency of stimulation above about 30 per second a smaller contraction resulted if the stimulus was only slightly supramaximal, but upon increasing the strength of stimulation the original level was once more restored. This effect is exactly similar to that observed in the preganglionic fibers and is even more readily obtained with the postganglionics. This may be associated with their higher threshold of stimulation, as Veach and Pereira suggested.

With both pre- and postganglionic stimulation there appeared at times a decrease in contraction at the higher frequencies of stimulation which was not prevented by increasing the intensity of the stimulus. This phenomenon suggests Querido's "maximal critical rate"; but we observed that it could never be obtained in fresh preparations and appeared only after a number of responses had been elicited or stimulation continued for a considerable length of time. There is some doubt as to the interpretation of this phenomenon. We suggest that the fresh preparation responds to a rapid rate of stimulation (100 or more) because impulses are set up in the nerve at a slower, perhaps irregular, rate. The failure of a preparation to continue to respond to stimulation at high frequency might be due to polarization at the stimulating electrodes. Even though our neon tube

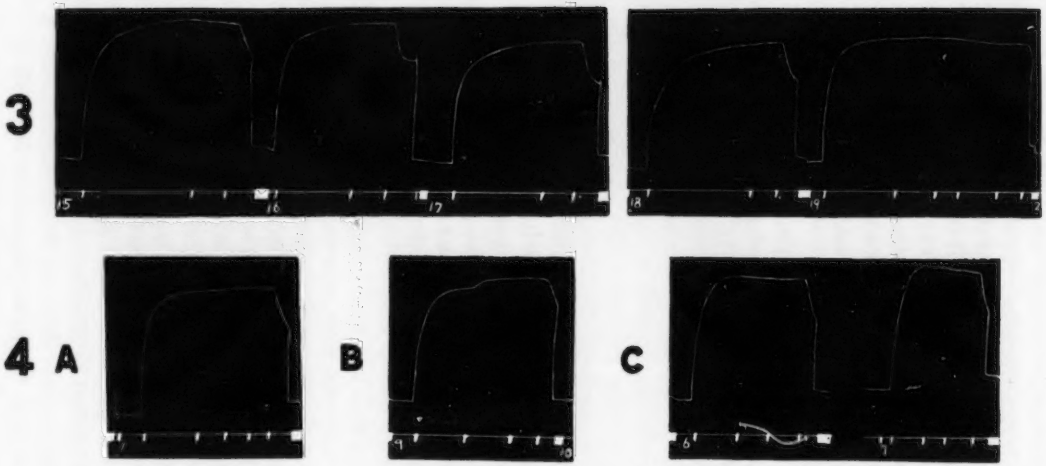

Fig. 3. 5/6/31. Preganglionic stimulation. First curve (15); 30 per second. At signal, stimulus increased from 7.5 to 8.7 times threshold. Second curve (16) at 70, third curve (17) at 100, fourth curve (18) at 200 per second. Otherwise similar. The atypical slow rate of contraction in the fourth curve indicates that the actual frequency of impulses set up in each fiber is probably much lower than that of stimulation.

Fifth curve (19); frequencies 50, 70, 100, 200 per second followed by increase in intensity as in first curve.

Fig. 4. Postganglionic stimulation.

A. 5/21/31. (1) Frequencies of 190, 136, 98 and 75 per second. Shifts of frequency and end of stimulation marked by signals. Intensity 6 times threshold.

B. 5/21/31. Frequencies of 10, 20, 30 per second. Intensity 6 times threshold.

C. 6/9/31. First curve, preganglionic stimulation at 190, 115, 75 per second. Intensity, 15 on potentiometer which is 5 times threshold.

Second curve, same preparation, postganglionic stimulation at same frequencies. Intensity 20 on potentiometer scale which is 5 times threshold.

stimulator is so arranged that the slow charging current of the condensor flows through the tissue in reverse direction to the stimulating discharge, this may not completely prevent polarization due to rectifying effects at the metallic surface of the electrodes or at the nerve sheath or within the nerve itself. With rapid stimulation such effects, although negligible at slower frequencies, might become important and serve to produce a persistent cathodal depression. This is analogous to the explanation invoked by Erlanger and Blair (1931) for the Wedensky inhibition. We did not control this possibility, but we think it more probable that the failure of contraction is an expression of the "pseudo-fatigue" described by Orias.

In order to confirm our conviction that Querido's results on postganglionic stimulation were due to the failure of fibers for some reason to respond to barely "maximal" stimulation, we performed the following experiment. The postganglionic fibers were stimulated at a rate of 150 per second with definitely supramaximal stimuli, and it was demonstrated that a maximal contraction of the membrane was maintained under these conditions. The intensity of stimulation was then reduced somewhat, although still above the original maximal value, and the frequency varied. Tracings much like Querido's were obtained, the contraction falling off when the frequency was raised and increasing when it was lowered. On once more increasing the stimuli to supramaximal intensity a maximal contraction at all frequencies was again obtained. This confirms Veach and Pereira's observations on this point.

DISCUSSION. We believe that these experiments demonstrate that Querido's "maximal critical rate" for the postganglionic fibers, which plays so large a part in his theoretical argument, is illusory and rests on the technical error of using inadequate strength of stimulation. Neither he nor Veach and Pereira reckoned with the possibility that if impulses are set up by rapid repetitive stimulation, their frequency may be lower than the frequency of stimulation. In our experiments we, like Bishop and Heinbecker, find no evidence to indicate that the frequency of impulses in the postganglionic fibers is different from that in the preganglionic fibers. Critical re-examination of Querido's results in the light of subsequent work likewise discloses no such evidence, unless possibly it be in his experiments on the effect of cooling the ganglion and the nictitating membrane respectively. The case here is so complicated that hypothetical explanations are not worth while, unless the experimental observations can be repeated with due precautions as to strength of stimulation, etc.

The failure of rapid frequencies of stimulation to maintain contraction even when there is no question as to the adequate strength of the individual shocks raises the question of fatigue. Our observations on this point are incidental, as we seldom stimulated continuously for more than two minutes, but on the whole confirm those of Orias (1932), who found that a rather

scale which is 5 times threshold.

rapid falling off of the contraction of the membrane occurred within 2 or 3 minutes when the preganglionic fibers are stimulated at the rate of 42 breaks per second. In our experiments, such a fall was most frequently encountered with stimulation frequencies of 70 or more, which suggests that in Orias' experiments the make shocks were effective, at least on the preganglionic fibers, as well as the breaks. Our observations differ from those of Orias in that we observed a similar fall in contraction on stimulation of the postganglionic fibers at the same frequencies, while he found that contraction was always well maintained on postganglionic stimulation. We are not prepared to offer a complete explanation of this difference, but merely note once more that in both sets of experiments there is no proof that the frequency of impulses in the nerve fibers was identical with that of the stimuli, and that the postganglionic fibers may differ from the preganglionic in their response in such a situation.

Orias further found that stimulation at 3 breaks per second would maintain the contraction, whether the stimuli were applied to the preganglionic or to the postganglionic fibers, and he points out the similarity of this greater effectiveness of slower frequencies of stimulation to certain of Wedensky's early observations and to similar observations on the soleus muscle by Davis and Davis (1932). Without attempting a theoretical explanation of this "pseudo-fatigue" he ascribes its locus to the superior cervical ganglion.

We believe that the locus of the phenomenon is still an open question, and suggest that it may rather be in the muscle. Davis and Davis observed that when the circulated soleus muscle is stimulated indirectly for several minutes, greater tension is maintained with a slower rate of stimulation and less with a more rapid rate. The size of the action currents recorded simultaneously indicated that with rapid stimulation the muscle did not at any time completely recover from the effects of the successive impulses, and it was inferred that, as a result of this incomplete recovery, the contractile energy liberated by each impulse was small and the resulting level of contraction low. Interpreting the phenomena in the nictitating membrane as analogous to those in the soleus, we suggest that the "pseudo-fatigue" presumably involves the process in the smooth muscle responsible for the initial potential of the action current described by Rosenblueth, Leese and Lambert (1933). If we adopt the view that the contraction in smooth muscle depends upon the formation of a chemical mediator and further assume that the formation of the mediator depends upon the process which gives rise to the initial electrical potential, then it is reasonable to suppose that the secretion per impulse will be smaller when the tissue is incompletely recovered. If the secretion per impulse is reduced more than enough to be offset by the greater number of impulses per second, a sufficiently rapid rate of stimulation would cause a smaller contraction than a slower rate. If definite evidence of the applicability of this principle to

smooth muscle can be obtained, it may aid in the explanation of various other inhibitory effects in smooth muscle which have been attributed to "Wedensky inhibition" (cf. Veach, Schwartz and Weinstein, 1930, on "vagal gastric effects").

SUMMARY

The contraction of the nictitating membrane of the cat has been studied in relation to the frequency of stimulation of its preganglionic and postganglionic nerve fibers. In both cases contraction is more rapid the greater the frequency of stimulation up to at least 200 per second. With definitely supramaximal stimuli, 10 times threshold strength, a steady contraction is maintained in fresh preparations for at least half a minute throughout this range of frequencies.

The extent of contraction increases with increase of frequency up to 25 or 30 per second and remains constant for higher frequencies, except that if the strength of stimulation is only slightly supramaximal, 5 or 6 times threshold, the contraction in response to frequencies above 30 per second may be submaximal from the start or may fall from maximal to a lower level. In fatigued preparations such a fall may occur even with stronger stimuli. The higher the frequency of stimulation, the easier it is to obtain this effect. Theoretical explanations of these phenomena are suggested.

When due allowance is made for different thresholds of stimulation, we find no consistent difference between the preganglionic and the postganglionic fibers in relation to frequency of stimulation. Our experiments do not support the hypothesis that the frequency of impulses in the postganglionic neurones differs from that of the impulses arriving over the preganglionic fibers.

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THE ACTION OF INSULIN ON THE MOTILITY OF THE EMPTY STOMACH

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In 1924 Bulatao and Carlson reported that the subcutaneous injection of insulin into normal fasting dogs increased the motility of the stomach. The increase in gastric tonus and in height and frequency of the contractions usually appeared in 50 minutes, developing into gastric tetany in one hour after injection. On injecting 20 to 40 units of insulin subcutaneously into the diabetic dog there was at first no influence on the gastric tonus and hunger contractions. Fifteen to twenty minutes after the insulin injection the gastric tonus began to decrease, and the hunger contractions became gradually weaker with longer intervals of rest, until at 30 to 60 minutes following the insulin, the stomach became relatively atonic and quiescent. This quiescence may last from 10 to 20 minutes, when the hunger contractions and gastric tonus return gradually and the typical gastric tetany of hypoglycemia appears 60 to 80 minutes after insulin administration. They stated that the primary action of insulin on the gastric tonus and hunger contractions is inhibition and that the gastric tetany of hypoglycemia comes later.

Quigley, Johnson and Solomon reported that the subcutaneous injection of insulin usually produced in normal fasting humans an increase in gastric motility. Templeton and Quigley reported that intravenous or subcutaneous injection of insulin inhibited the motility and depressed the tone of a Heidenhain pouch. Quigley and Templeton reported that insulin inhibited gastric hunger contractions in dogs having the vagi sectioned. Simici, Guiera and Dimitriu reported that the injection of insulin in normal fasting men inhibited gastric motility. Mulinos reported similar results on dogs.

METHOD. Five dogs, weighing from 13 to 17 kgm., with gastric fistulae, were used. These dogs were trained to lie quietly during the experiments which were from 3 to 9 hours' duration. All experiments were performed after at least 18 hours' starvation. The dogs were kept on a constant diet and were in good condition.

The gastric movements were recorded by means of a rubber balloon attached to a water manometer. The balloon was inflated with a

constant amount of air. A flexible wire connected to a spring was placed within the balloon in order to keep it from moving around in the stomach. This device enabled one to get constant records from day to day. The balloon was placed in the pyloric end of the stomach and anchored there. The insulin used in these experiments was the "Iletin" of Eli Lilly & Company.

Records were run on the dogs until the normal was established before beginning to inject insulin. Before each injection a control record was run for from 2 to 6 hours The insulin was injected either intravenously or subcutaneously in doses of 2 to 30 units. The record was continued without interruption until the animal became very restless or went into convulsions (usually about $1\frac{1}{2}$ to 2 hours after insulin) in which event glucose was administered intravenously or into the stomach. The glucose was given in the form of a 30 per cent solution and was given in amounts of 4 to 40 grams.

Injections were made during the height of gastric motor activity and in quiescent periods. Ringer's solution or physiological saline was injected

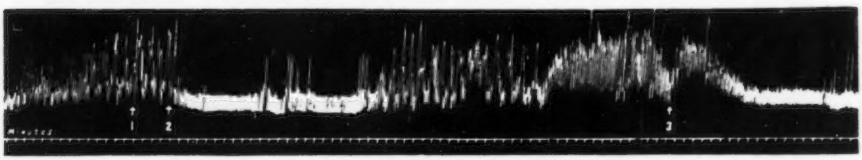

Fig. 1. Dog fasting 18 hours in normal gastric hunger period. *1*, injection of 1 cc. Ringer's solution intravenously. *2*, injection of 20 units insulin intravenously. *3*, introduction of 30 grams glucose into the stomach through the gastric fistula.

preceding the insulin in order to rule out the effect of the injection itself. Blood samples were taken and blood sugar determinations made. These results will be published as soon as sufficient data are accumulated.

Results. When 10 to 20 units of insulin were injected subcutaneously into normal dogs during a hunger period, there was at first no influence on the gastric tonus and hunger contractions; a few minutes after the insulin injection the gastric tonus began to decrease, and the hunger contractions became gradually weaker with longer intervals of rest until, on the average 18 minutes following the insulin, the stomach became relatively atonic and quiescent. This quiescence may last for from 15 to 60 minutes, when hunger contractions and gastric tonus return gradually.

On intravenous injection of from 2 to 30 units of insulin into normal dogs during a hunger period there was a fall in tone and quiescence within one minute after injection. Generally the stomach contracted once after the injection and then became quiet. This quiescent period lasted from

3 to 45 minutes depending upon the dosage, when contractions and gastric tonus return. This activity resembles very closely type III gastric activity as classified by Carlson. The dogs then became very restless, salivated, whined and sometimes went into convulsions. The intravenous injections of glucose relieved these symptoms and caused a gradual fall in tone and a diminution of activity followed by quiescence.

In order to determine whether or not this inhibition was due to impurities in the insulin preparations, we obtained some crystalline insulin from the Connaught Laboratories. The crystals were dissolved in hydrochloric acid at a pH of from 2 to 3. We injected 0.01 N hydrochloric acid as a control. There was no effect shown on the tracing. The injection of from 2 to 20 units of the crystalline insulin intravenously produced the same type of response as the commercial preparation.

Mulinos has reported that the failure of insulin to produce gastric hyperactivity was due to the lack of some dietary factor, probably vitamin B. It was thought that this might be the cause of the inhibition following intravenous injection of insulin. Two dogs, therefore, were fed 25 grams of fresh yeast daily along with their regular diet of bread, cooked meat and milk for one month. No detectable changes were recorded on the tracings.

In order to rule out the action of the insulin preservative 0.2 per cent phenol in 0.01 N hydrochloric acid was injected intravenously. No effects on gastric motility were observed.

Discussion. Although this inhibition was not reported by Carlson or Templeton and Quigley on subcutaneous injection, this is probably due to the fact that they considered the cessation of activity as the end of a normal active period. It is probable that the continued but diminishing activity after subcutaneous injection of insulin is a latent period, i.e., the time necessary for the insulin to be absorbed in sufficient quantity to inhibit the activity of the stomach. It is possible to keep the animal's stomach in a state of quiescence for a considerable time by giving repeated doses of insulin. The successive doses were given at the beginning of the tone change.

These results also explain what was thought to be a discrepancy in the effect of insulin on gastric motility in normal and in pancreatectomized dogs. Bulatao and Carlson reported that the injection of insulin into normal fasting dogs increased the motility of the stomach and that in diabetic dogs insulin produced a primary depression of gastric tonus and contractions, followed by increased gastric tonus and contractions when the initial stage of hypoglycemia was reached. The latter has been shown to be the normal reaction on injection of insulin.

It appears that the intravenous injection of insulin stimulates the sympathetic nervous system. There is a fall in tone and a quiescence of the

stomach, accelerations of the heart rate, sweating, constriction of the peripheral blood vessels and dilatation of the pupils in man (see also Cannon, McIver and Bliss). Dworkin has reported that in normal cats and those surviving excision of various portions of the autonomic system the injection of insulin produced the following changes in the heart rate: 1, acceleration due to central sympathico-adrenal impulses; 2, slowing, due to central vagus stimulation; 3, slowing, due to a peripheral action. He has also reported that in the intact animal these changes are expressed in a heart rate that is seldom above the basal level. This is in accord with the work of Templeton and Quigley who reported that the intravenous or subcutaneous injection of insulin inhibits the motility and depresses the tone of a Heidenhain pouch. They reported that the action of insulin in double splanchnicotomized dogs while fasting is similar but slightly greater that that displayed by normal animals. The quiescence continues until the blood sugar falls sufficiently low to cause central parasympathetic stimulation, as is shown by the initiation of gastric motility, slowing of the heart rate, salivation, and constriction of the pupils. If, now, according to Dworkin, both divisions of the autonomic nervous system are stimulated during the insulin hypoglycemia, the parasympathetic system predominates in its influence on the various organs until the convulsive sugar level is reached. This is supported by the facts that insulin inhibits gastric hunger contractions in vagotomized dogs while intravenous injection of glucose is practically without effect on the motility of such a stomach and that the injection of atropine will inhibit the activity of the stomach following insulin.

SUMMARY

1. The intravenous injection of insulin in normal dogs produces a primary depression of tonus and contractions of the stomach followed by an increase in tonus and gastric activity which passes into a regular type III activity, or incomplete gastric tetany.

2. This is a true insulin effect because the same type of response is obtained on injection of crystalline insulin preparations.

3. The insulin response in normal dogs is identical to that reported for pancreatectomized dogs.

4. The increased sympathetic activity and increased adrenalin output after insulin (Cannon, McIver and Bliss) have been correlated with the increased gastric activity (Bulatao and Carlson).

The writer is greatly indebted to Dr. D. A. Scott of the Connaught Laboratories, Toronto, for supplying the crystalline insulin used in these experiments and to Dr. A. J. Carlson under whose direction this work was completed.

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STUDIES IN THE HUMAN NEURO-MUSCULAR MECHANISM

I. The Theory of "Subsidence of Afferent Flow" as an Explanation of the "Lengthening Reaction" and Other Phenomena[1]

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It is becoming increasingly apparent that a transfer and adaptation of known facts in animal neurophysiology must be made to human material before a complete concept of the physiology of human nerves and muscles and its clinical application will be possible. For some time we have endeavored to study cases of spasticity of human skeletal muscles in the light of knowledge gained from animal experimentation and have attempted by various procedures to alter the postural state of the skeletal muscles involved. In the course of our observations, especially of the "lengthening reaction," we have found difficulty in accepting the theory of autogenous inhibition as an explanation of some of the phenomena which we have observed. It is our purpose here to point out the difficulties in regarding autogenous inhibition as an explanation of the relaxation which is the "lengthening reaction" and to attempt to establish and illustrate another hypothesis which seems to us to be much more acceptable in the light of known facts.

Part I. The "lengthening reaction" is a readily demonstrable phenomenon both experimentally and clinically. It is essentially composed of two parts which are such constant concomitants of so-called pyramidal or decerebrate rigidity that clinically the phenomenon has resulted in this type of rigidity being termed "clasp-knife." It can be easily demonstrated by attempting to flex the leg of a hemiplegic or paraplegic patient. The attempt at first meets with marked resistance on the part of the extensor muscles. With increasing flexion (stretch of the extensor muscles) this resistance proportionately increases to a variable point at which it suddenly subsides allowing the leg to be further flexed with very little effort. If the flexing force is released the extremity will be found to have adopted this new position; in other words, the muscle remains quiescent at its new length. These two phenomena, the sudden subsidence of tension and the assumption of the new length imposed upon the muscle, are the "lengthening reaction," or clinically constitute "clasp-knife" rigidity.

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This effect has commonly been interpreted as due to inhibitory impulses, arising within the muscle and its tendinous attachments. These impulses have been thought to arise from sensory end organs, possibly nociceptive in type, which act as a protective mechanism against rupture or injury of the muscle and its attachments. These inhibitory impulses must be considered as lowering the central excitatory state during the period of excessive stretch. As the muscle is stretched, so as to produce the "lengthening reaction," it responds with increasing tension to a maximum from which it rapidly subsides to a much lower but constant level, which, although the muscle remains at the increased length for long periods of time, remains far below the maximum tension developed. Thus an inhibitory influence, if present, must persist throughout the period of stretch. It is conceivable that the supposed inhibition might be developed either in response to the actual lengthening of the muscle, *i.e.*, the stretching, or in response to the maximum tension developed by the muscle. It would seem that the latter hypothesis does not agree with the fact that the "inhibitory" influence persists long after the tension has subsided. If inhibition were in response to the tension the inhibition would induce a relaxation, but with the subsidence of tension the inhibitory effect would be removed and following a brief period of after-discharge the maximum tension would reappear because the stretch is still present. As this does not occur we must look for some factor which is present at the initiation of relaxation and persists subsequently. The most obvious thing which fulfills these requirements is the continued stretch. Thus if inhibition is present it must arise in response to a constant factor such as the excessive stretch, and not to one only momentarily present as the maximum tension.

This statement that the "inhibitory" influence must persist throughout the period of stretch after the initiation of relaxation is, of course, contrary to the work of Sherrington (11) who found that there was *no* alternation in the central excitatory state after the "lengthening reaction" had occurred, *i.e.*, after the muscle had assumed its new length and therefore concluded that the duration of the inhibition was only that of the forced stretching.

In addition Denny-Brown (1) has shown that the quiescence of efferent impulses, which results in relaxation and which he attributes to autogenous inhibition occurs *only* in response to *an efferent volley of impulses*, that is in response to muscular contraction and therefore must be in response to muscular activity and not to stretch itself.

Thus if inhibition cannot be produced by either the stretch itself or the resulting mechanical tension it would seem necessary to discard autogenous inhibition as an explanation of the "lengthening reaction." However, it should be clearly understood that failure of the theory of autogenous inhibition to explain the lengthening reaction does not abolish the possibility of

inhibitory impulses arising in response to actually endangering tensions and protecting the integrity of the muscle and its attachments.

It might be well to examine the factual basis upon which this thesis of autogenous inhibition rests. The evidence for the reflex nature of the "lengthening reaction" has been summarized by Fulton (5). These facts are very acceptable as evidence that we are dealing with a true reflex but as we shall point out are no proof that we are dealing with reflex inhibition.

1. "It (the 'lengthening reaction') occurs in the fully isolated quadriceps muscle." This fact demonstrates only that the relaxation, like the contraction, originates within the muscle itself. As to the cause of the relaxation it offers no information and any other explanation which is acceptable on the basis of an isolated muscle would serve as well.

2. "It is graded in intensity as is the shortening reaction." This, of course, is no argument for the existence of inhibitory impulses but only evidence that whatever effects the relaxation is in close association with the contraction and, might we not add, with its afferent mechanism.

3. "It is not to be elicited in a de-afferented muscle" and 4, "It disappears under light anesthesia as with other reflexes." In view of the fact that the "lengthening reaction" is relaxation, and there must exist contraction before relaxation can occur, any factor which abolished the stretch reflex would automatically abolish even the possibility of a lengthening reaction regardless of its cause.

5. "Being in the nature of a relaxation, it is similar to such inhibitory relaxations as are produced by stimulation of the central end of an ipselateral afferent nerve." For many reasons this argument is no proof that the relaxation owes its origin to autogenous inhibition. First, because there is a vast essential difference between the two phenomena. In the one we have an actual artificially produced stimulation of an afferent nerve with inhibitory effects. In the other we have at present absolutely no evidence of any such stimuli, nor of their ability to produce inhibition even if present. And in this fact lies the great weakness of the thesis of "inhibition" as an explanation of the "lengthening reaction." The fact that electrical or mechanical or other artificial stimulation of an afferent nerve either arising from the muscle itself or otherwise, is capable of inhibiting the stretch reflex does not for a moment prove that in the intact muscle such stimulation and inhibition occur. It may be considered to demonstrate a pathway which is capable of bearing inhibitory impulse if and when suitably stimulated. Further than that it does not go.

6. "When the muscle is palpated and the 'lengthening reaction' evoked the feeling is in every way similar to that which occurs in response to reflex inhibition." This is, of course, only evidence that the relaxations induced by the "lengthening reaction" and by known inhibitory impulses are com-

parable on palpation and no evidence that the relaxation of the "lengthening reaction" is secondary to autogenous inhibitory impulses.

In addition Fulton (6) has advanced three other phenomena as proof that the "lengthening reaction" is produced by autogenous inhibition. He states, "I conclude that inhibition may be present in the 'lengthening reaction' because I have frequently seen the following phenomena during its elicitation:

"1. Crossed extensor reflexes on the opposite side (hind limb) and ipselateral extension of the forelimb.

"2. Flexor contraction of the hamstrings of the same limb.

"3. Concomitant inhibition of synergic extensors of the same limb. (see also Denny-Brown (1)).

"This in my (Fulton's) opinion proves that autogenous inhibition exists and that in the presence of dangerous tension, may become a part of the reflex protective mechanism. It does not prove that such a mechanism operates in the normal postural adjustments occurring in the intact animal or in states of spastic contraction."

Let us briefly examine these three facts. The first two are definitely only direct evidence of effector impulses and in no way evidence of inhibition. The third is certainly probable evidence of the presence of inhibitory impulses. However, this must be accepted only with reservations because the direct evidence is only that of the arrest of efferent impulses, the proof that this is due to inhibitory afferent impulses and not to the arrest of excitatory afferent impulses is lacking. In addition, even though we believe it to be due to inhibition we must admit that the evidence is only that of inhibition of other muscles and only very indirectly can it be considered as evidence of *autogenous* inhibition.

Although autogenous inhibition may develop as a protective mechanism against dangerous tension there is then no good evidence that it is the sole or even a major factor in the production of relaxation which is seen in the ordinary response of the muscles to stretch or in the "lengthening reaction." Thus the evidence at hand leaves us with the following conclusions: 1, the stretch reflex can be inhibited by artificially induced impulses over various afferent nerves from that extremity, and 2, there exist pathways from the muscle itself, and from other tissues, capable of bearing inhibitory impulses. But there is no evidence that autogenous inhibitory impulses exist and produce the "lengthening reaction." It would seem that the proof of this point and an explanation of the absence of any alteration of the central excitatory state during the period of relaxation after the heightened tension are essential to the proof of the thesis of autogenous inhibition.

PART II. If for the moment we can assume that the hypothesis of "autogenous inhibition" as the explanation of the "lengthening reaction" is lacking in sufficient proof we may be permitted to examine other possible ex-

planations. As the phenomenon may appear in response to stretch in a muscle completely isolated from all external influences and controls, except for its central nervous connections and the stretching force, and as the central connections may be assumed to remain in a static condition, except for the influence of the stretch reaching it by way of the muscle and its afferent pathways, it would seem only reasonable that the nervous mechanism must receive its initial impulse from within the muscle itself. It would seem that it can safely be assumed that in the "lengthening reaction" we are dealing with *relaxation* of the muscular contraction produced by the stretch reflex, and that the stretch reflex is muscular contraction which is effected by way of the anterior horn cells and their processes, which are in turn activated by afferent impulses originating within the muscle itself in response to stretching. Thus we visualize a reflex arc satisfactory unto itself and free from external factors. If now, for the sake of discussion, we eliminate the possibility of a self-induced inhibition there remains but one possible means by which the contracting muscle can be relaxed, so long as the stretching force is active. *There must occur a subsidence or a cessation of the afferent impulse reaching the reflex center.* Three groups of careful workers have demonstrated that this is exactly what occurs during active contraction. In 1928 Fulton and Pi-Suñer (7) demonstrated that the irregular action currents present in extensor muscles under tension, in decerebrate rigidity, and which are conceded to be the electrical manifestations of the proprioceptive (or stretch) reflex on the motor side, disappear during the contraction of the muscle in the knee-jerk. This subsidence of action currents they attributed to a subsidence of afferent impulses during the phase of contraction. They explained this interruption of afferent flow during the continuance of stretch by assuming that the muscle fibers and the afferent end-organ were arranged "in parallel" rather than in series. In other words, that when the muscle fiber contracted it relaxed the tension upon the end-organ and thus the stimulus no longer being present, the end organ ceased to fire off and in turn the action currents in the muscle ceased.

This hypothesis was soon subject to criticism by Denny-Brown (1). He stated that this hypothesis depended upon actual shortening of the muscle, else the tension upon the end-organ would not be relieved. And as the phenomenon occurred in the absence of shortening the hypothesis must be untenable and he suggested in its place that the added tension occasioned by the contraction of the knee-jerk gave rise to inhibitory impulses which arrested the irregular action currents in the hypertonic muscle. This thesis was, of course, in accord with the Sherrington school in regard to "autogenous inhibition" and fits well with the "inhibition" explanation of the "lengthening reaction." It was, however, destined to be very short lived. Matthews (9) in 1931 in a brilliant piece of work attacked the problem from the other (afferent) side of the reflex arc. He has very con-

clusively demonstrated that the afferent impulses occasioned by stretch disappear during the active phase of muscular contraction and reappear with relaxation of that contraction. During the active phase of muscular contraction there exist *no* afferent impulse (of either excitatory or inhibitory character). He also very simply disposed of Denny-Brown's objection to Fulton and Pi-Suñer's hypothesis by slightly altering the hypothetical model of the arrangement of muscle fibers and end-organ. Thus if one supposes the common point of attachment of muscle fiber and end-organ (arranged in parallel) to be slightly elastic it no longer becomes necessary for any shortening of the muscle to occur in order for contraction to relieve the tension of stretch upon the end-organ.

Several years ago Forbes, Campbell and Williams, in a study of the afferent impulses from muscular receptors in the tibialis anticus of the cat, determined that during muscular contraction, induced by a single break-shock, there occurred two volleys of afferent impulses. The first coincided with the onset of contraction, and the second occurred during the period of relaxation, a measurable period of time after the peak of the contraction, but during the major part of the contraction, *i.e.*, during the period of highest tension there was little evidence of afferent impulses.

On this evidence alone it cannot be definitely stated that there were *no* afferent impulses arising from the muscle during the period between the two volleys. But it is obvious that there were no synchronous volleys of impulses and no asynchronous impulses sufficient to cause appreciable deflection of the string of the galvanometer. This work thus agrees in the main with that of Matthews.

Thus there have been adequately demonstrated two points essential to our hypothesis: 1, active contraction of the muscle abolishes the constant irregular action currents present in the stretched muscle of a decrebrate animal; 2, active contraction abolishes the afferent impulses produced by stretch of a muscle.

These leave us with but one conclusion, that the "lengthening reaction" is produced by a subsidence or cessation of the afferent impulses arising from the muscle secondary to stretch and is not occasioned by inhibition. In other words, the muscle is stretched, and in response it contracts but in contracting it relieves the tension on the end-organs, thus causing the afferent flow to cease, and the contraction subsides.

An obvious criticism immediately confronts us. As soon as the muscle relaxes, tension again plays upon the end-organ and contraction should recur. And as a matter of fact this is exactly what happens. If, as is often the case some degree of relaxation occurs long before the completion of stretch the subsequent contraction may be much greater than the initial one because stretch is increasing, the stimulus is greater and thus the response is greater. At times two or three such relaxations and contrac-

tions may be observed before the active progressive stretching is terminated. And if the central excitatory state is great enough, the initial tension may continue with little decrement after the stretch becomes static, and clonus may develop. However, in the majority of cases clonus does not ensue. Ordinarily the contractions rapidly become weaker until a state of equilibrium between relaxation and contraction (or between the muscle fiber and the end-organ) occurs and a constant state of contraction at a much lower tension than that originally produced results.

PART III. It may prove of advantage, in elucidating this hypothesis, to attempt its application to various phenomena observed in human skeletal muscle.

Obviously the physiological study of human muscle is fraught with many difficulties and pit-falls. It is not possible to isolate individual groups of muscles thus freeing them from the possible effect of other muscle groups, afferent innervation from superficial tissues, etc., or to provide ourselves for study with sharply demarcated, acute lesions of the nervous system. Nor has it proved feasible, as yet, completely to immobilize the part, connect the muscle with an isometric myograph and record alterations in electrical potential from efferent and afferent nerves. Nevertheless it is becoming increasingly apparent that, for the correct interpretation of clinical findings in accordance with the vast mass of experimental facts obtained by animal experimentation, an attempt must be made to transfer information true for the neuro-mechanism of lower animals to human neuro-physiology. There is increasing evidence that the reaction of the nervous system of cats and dogs is not identical with the reaction of monkeys, much less of man. We have only to cite the difference in the response to spinal transection to illustrate this point (8). It becomes apparent that no known fact in animal neuro-physiology can be stated to be completely true in human physiology until experimentally proven.

METHOD. It has been our endeavor to attempt to interpret the findings in human cases of spasticity of skeletal muscle in the light of experimental findings. This discussion of the lengthening reaction is the outgrowth of our observations made upon human cases. In studying the responses of human skeletal muscle we have utilized the apparatus designed by Schaltenbrand (10). This method has numerous disadvantages which we have attempted to bear in mind at all times, but it seems permissible not to enter into a discussion of them at this time since our observations are in no way offered as proof of our thesis but only to illustrate it.

The tracings to be presented are from three cases, all children, who are suffering either from congenital spastic paraplegia or quadriplegia. In one case which we shall refer to as case 1, a boy of sixteen with a paraplegia, the tendons of the flexors of the knees had been severed at an early age and the operation so efficiently performed that these muscles were functionless

so far as could be observed. In case 2, a girl of ten, there is a severe completely incapacitating quadriplegia and there have been no sections of tendons about the knees. In case 3, a girl of ten, also quadriplegic, there is far greater involvement of the lower than of the upper extremities and tenotomies had been performed on the flexor muscles of the knee on both sides, rendering them almost functionless.

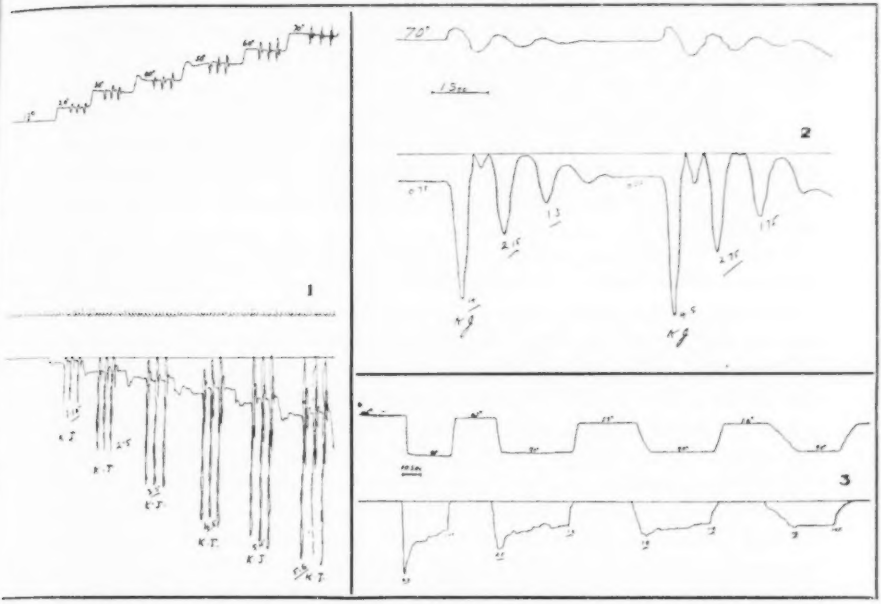
All observations have been made upon the extensor muscles of the knee. In the particular observations cited the patients have been fully conscious, coöperated voluntarily, and no anesthetic of any kind was employed except as noted. The tracings reproduced here have been selected from repeated observations and could be duplicated from many similar examinations.

Observations. 1. As has previously been stated Sherrington demonstrated that there is no alteration in the central excitatory state, *i.e.*, there is no inhibition in the period during which stretch of the muscle is continued after the relaxation of the "lengthening reaction" has occurred. We wish to present an observation in support of this evidence.

If "inhibition" persisted after the relaxation and if relaxation were produced by inhibitory impulses developed secondary to stretch of the muscle, then increased stretch should give rise to increased "inhibition," thus lowering the central excitatory state in proportion to the degree of stretch. In view of Sherrington's findings this is, of course, not anticipated, nor is any evidence of it found. If we examine the mechanical response of the muscle to percussion of the patellar tendon (knee-jerk) we invariably find that with increased stretch of the muscle there is a consistent increase (within variable limits which at the present need not concern us) in the mechanical response (fig. 1). Because the explanation of this progressive enhancement of the knee-jerk (stretch reflex) is not closely related to the matter at hand, it may be dismissed for the moment to avoid confusion. But it gives definite evidence that there is no increase in inhibition of the central mechanism with increased stretch of the muscle. As we have previously considered the question of the presence or absence of "inhibition" after the relaxation of the "lengthening reaction" and its possible significance, we need also not discuss it here.

However, the tracing brings up another point. It will be observed that there is an unusual uniformity of the mechanical response with the muscle stretched to any given length. This is not peculiar to this one observation but has been observed on many occasions. There was no effort made, and no apparatus used, to obtain blows to the patellar tendon always of the same strength and always delivered to the same point. An ordinary percussion hammer was used. It seems possible, therefore, to assume that the stimulus in each instance was maximal or supra-maximal in intensity. It might further be stated that reversing the procedure, that is, starting with the greater degree of stretch first, does not alter the results.

2. It is to be anticipated on the basis of our hypothesis that the rapidity of stretching would alter the resultant mechanical response of the muscle.



In all figures the uppermost tracing indicates the angulation of the leg upon the thigh. The figures (13°, 20°, etc.) indicate the degree of flexion away from complete extension, *i.e.*, at 90° the leg is at right angles to the thigh. The middle tracing indicates time at intervals of one second. The lowermost tracing is composed of two parts, a base line representing zero and a second line indicating the amount of mechanical tension developed in the extensor muscles of the thigh. The figures are indicative of relative tension as determined by previous calibration of the apparatus.

Fig. 1 (reduced about ½). Case I. Mechanical response to percussion of the patellar tendon. At 13° of flexion there was no measurable tension in the extensor muscles (*i.e.*, any tension in the extensors was exactly counterbalanced by a flexing force and the extremity was "at rest"). With flexion to 20° a slight tension developed, which subsequently increased with each additional stretch (flexion). The mechanical response of the knee jerk progressively increased with each added stretch.

Fig. 2 (reduced about ½). Case I. The leg was flexed to 70° developing a sustained tension ("equilibrium") of 0.75 unit in the extensor muscles. The patellar tendon was then percussed with an ordinary clinical percussion hammer and 4 units of tension in one instance, 4.5 in the second developed. The secondary oscillations may be dismissed for the moment.

Fig. 3 (reduced about ¾). Case I. Showing the effect of the rapidity of stretch. The more rapidly the stretch is developed the greater the initial response. When stretched sufficiently slowly as in the fourth tracing the tension is not initially greater than when sustained. The constancy of the "sustained" tension regardless of the rate of stretch is striking.

In the muscle at rest the muscle fibers are relaxed thus "exposing" the end organs to the full force of any stretch. If the stretch is developed rapidly it suddenly exerts its influence upon a large number of "exposed" end-organs thus producing a sudden volley of afferent impulses which provoke a correspondingly strong mechanical response. The ultimate result of this is seen in the knee-jerk in which a strong mechanical force is brought to bear upon the "exposed" end-organs in a very short space of time and with relatively little stretch (fig. 2). As a result there is a single strong contraction of the muscle, far out of proportion to the stretch, for the reason that the "exposed" end-organs are particularly susceptible to such a strong, suddenly applied force.

If the stretching force is still applied quickly, although far slower than by percussion of the tendon, by rapidly flexing the leg upon the thigh, a strong contraction results which more or less quickly subsides to "equilibrium" between the contracting fibers and the end-organ. This is the "lengthening reaction." The subsidence is quick because the contraction is both rapid and strong. In other words, a rapid stimulation of a large number of end-organs occurs resulting in a sudden strong contraction, which in turn relaxes the stretch upon a large number of end-organs and permits a rapid subsidence of tension. If the stretch is somewhat slower it first stimulates a certain number of muscle fibers. These, if the stretch is just slow enough, contract sufficiently to relax the tension on a few of these end-organs before the progressing stretch has had time to recruit others, and so on throughout the stretching. As a result the entire fused result of the contraction of the various muscle fibers is less than occurs when the stretch is carried out more rapidly. Thus in turn the subsidence to "equilibrium" is slower, which is due to the fact that, as relaxation occurs, certain of the end-organs earlier relaxed, *i.e.*, before consummation of the stretch, are now functioning, producing a certain degree of contraction and retarding relaxation. It thus becomes apparent that if stretch is executed slowly enough no major contraction would result and tension would slowly develop to the state of "equilibrium" which would be achieved at the termination of stretch. "Equilibrium" can be defined as that state in which the contraction of the muscle fibers is just sufficient to permit the proper tension upon a sufficient number of end-organs to maintain a certain definite degree of contraction in the muscle fibers.

In accordance with the preceding suppositions it may be seen in the accompanying tracings (fig. 3) that a rapid stretch produces an initial response stronger than that subsequently maintained, and that the degree of initial response depends on the rapidity of stretch. Further, if the stretch is applied sufficiently slowly, although quite steadily, there is no initial response that is greater than that subsequently maintained. Finally it may be seen that "equilibrium" is achieved at the same approximate

tension no matter what the rate of stretching may have been; stated differently, the initial contraction preceding the "lengthening reaction" varies directly in intensity with the rapidity of stretch, the relaxation which constitutes the "lengthening reaction" also varies directly in rapidity of development with the intensity of the initial response and the sustained tension, which persists after the relaxation, is relatively constant for the degree of stretch, not being influenced by the rapidity of stretching or the intensity of the initial response.

Thus we see that in the hypothesis of the "parallelism of the muscle fibers and the end-organ" we have a finely adjusted mechanism which adequately explains the phenomena of contraction and relaxation during stretch without invoking an additional and unestablished inhibitory mechanism.

3. If we now examine similar observations made upon faster moving tracings we shall be able to analyze them more closely and see how well the observed phenomena fit with the theoretical considerations. We have postulated that, unless the stretching developed extremely rapidly as in the knee-jerk, the increment of tension would not be a constant one, as in the case of the knee-jerk (see fig. 2), but would be interrupted by alternating contractions and relaxations. That this is true can be readily demonstrated by any one who will flex a hemiplegic patient's involved leg, and is very clearly shown in figures 4, 5, and 6. This phenomenon is readily explained upon the hypothesis of the "parallelism of the muscle-fibers and the end-organs" but is not so easily interpreted on the thesis of "autogenous inhibition." With the first hypothesis we may suppose that the relatively slowly developing stretch first stretches a small number of end-organs, which in turn cause a small number of muscle-fibers to contract, resulting in the first manifestation of tension in the muscle as seen in the tracing (*e.g.*, fig. 4). This contraction "protects" some, probably not all, of the originally stimulated end-organs because the stretch is steadily progressing, thus again stimulating these only partly "protected" end-organs and others not originally stimulated, so not "protected" by muscular contraction or "exposed." This results in a second and greater mechanical response. The process may proceed through a variable number of successive contractions and relaxations (four in fig. 6, and three in fig. 4), depending upon a number of factors, such as the degree and rapidity of stretch, the central excitatory state, the irritability of the end-organs and the muscle-fibers, etc. It continues to progress until the contraction is of such an intensity as to "protect" a sufficiently large number of end-organs, and reduce thereby the number of "exposed" end-organs to the point where the intensity of the afferent flow is not sufficient to maintain the contraction, then relaxation occurs. But, as can be clearly seen in all tracings, it does not occur suddenly or smoothly for relaxation again "exposes" certain end-

organs and alternating contraction and relaxation occurs until "equilibrium" is reached. In other words, we have at rest a muscle fiber—end-organ system in "equilibrium" which stretching disturbs; as soon as the stretching force again becomes constant at the same or a new level the system, set into oscillation by the stretching, continues to oscillate until it again arrives at "equilibrium." This oscillation is particularly well shown in figure 5.

Now let us examine the observations in the light of the theory of "autogenous inhibition." If we take figure 4 we find that the first contraction-

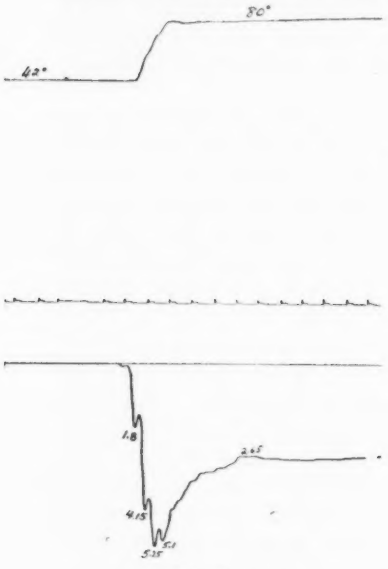


Fig. 4

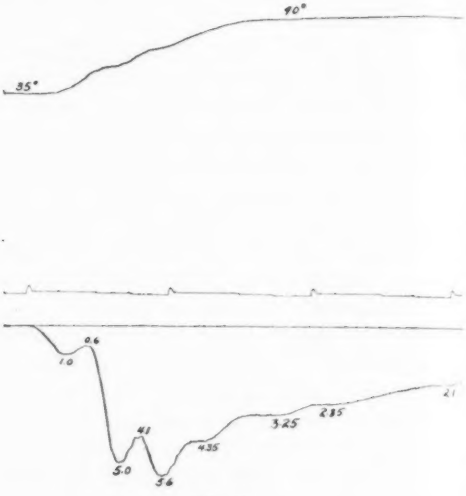


Fig. 5

Fig. 4 (reduced about ½). Case I. Fairly rapidly developing stretch resulting in an initial tension of 5.6, which is arrived at through three contraction-relaxation complexes. Relaxation or the "lengthening reaction" develops slowly and irregularly due to oscillation in the system and finally arrives at equilibrium at 2.65.

Fig. 5 (reduced about ½). Case II. This tracing is particularly valuable for the clear demonstration of the oscillation in the system before "equilibrium" was established.

relaxation complex developed with a tension of 1.8. We must, therefore, assume that a tension of 1.8 or that a stretch produced by flexion somewhere between 42° and 80° would be sufficient stimulus for such autogenous inhibitory impulses. But Sherrington has demonstrated that there is no inhibitory effect during the period of sustained tension, or "equilibrium," here developed at a tension of 2.65 and 80° of flexion. Both factors are

thus greater during the sustained tension than they were at the time of the first contraction-relaxation complex. Therefore, if a tension of 1.8, or less than the stretch of 80° of flexion, is sufficient stimulus for autogenous inhibition, certainly a tension of 2.65 or the stretch of 80° of flexion should be sufficient stimulus for such inhibition. Yet there is no inhibition in the latter instance. Can we reasonably assume that there is any in the first?

4. In figures 4 and 5 it will be observed that relaxation begins before the termination of stretch; such is also a common clinical observation. However, this is obviously not an essential part of the observation, for if

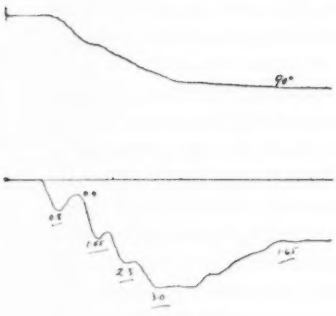


Fig. 6

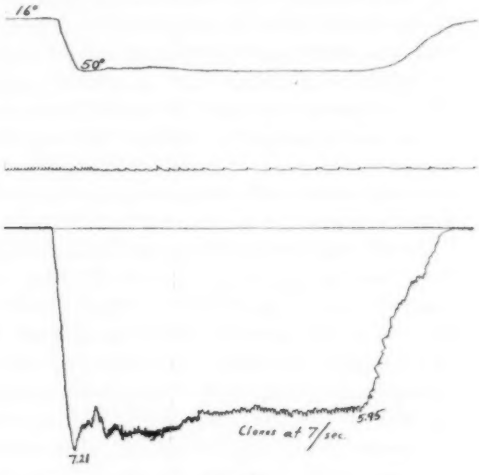


Fig. 7

Fig. 6 (reduced about ½). Case III. Similar to figures 4 and 5 except that stretching has been terminated during the period of maximum tension, thus demonstrating that it is not essential for relaxation (the "lengthening reaction") to have begun before the termination of the stretching.

Fig. 7 (reduced about ½). Case I. Flexion of the leg to only 50° produces a much more intense mechanical response than previously observed with greater flexion (*i.e.*, greater stretch of muscle, cf. figs. 3 and 4), indicating a greater irritability of the reflex system. "Equilibrium" was attained at a greater tension, both actually and in relation to the initial response than in other observations. At "equilibrium" there is a clonus, rhythmic contraction and relaxation, of small amplitude and at the rate of seven contractions per second. Note that the speed of the tracing was altered twice during the observation.

the progressive stretching were suddenly terminated at the apex of the last mechanical response the beginning of relaxation would then coincide with the termination of progressive stretching (fig. 6). Since relaxation can be seen to be a slower phenomenon than contraction it is not surpris-

ing that in the majority of tracings relaxation occurs before stretch is terminated.

5. Previously we pointed out that it is theoretically possible, if the irritability of the system is great enough, for only very slight relaxation to develop after the initial major contraction, and that "equilibrium" might be arrived at with the termination of stretch and clonus might be produced. In the following instance we were fortunate enough to observe this situation. The patient, referred to here as case I, was given a spinal anesthetic and as is common practice with such a procedure ephedrine was administered hypodermically to prevent the development of shock. There is no need to go into details as to the results of this experiment except to say that it established the fact that the mechanical responses which we have been observing in response to stretch were dependent upon intact nervous connections. As the patient recovered from the anesthesia he developed an excitability of the stretch-reflex far greater than was ever before or subsequently observed in this case. This we attributed to the administration of ephedrine but as we have had no opportunity to confirm this assumption we do not wish to stress it.

In the observation under consideration (fig. 7) the leg was flexed to only 50°; therefore much less stretch than was applied to the same case in obtaining the tracings shown in figures 3 and 4, where the leg was flexed to 90°. Yet 7.21 units of tension developed. Subsequently the tension subsided slightly arriving at "equilibrium" at 5.95, only a little less than the initial tension. However, the tension was not constant but was a typical clonus, *i.e.*, a rapid slight oscillation in contraction and relaxation at a rate of seven contractions per second. Unfortunately, as will be observed, the first part of the tracing is not clear. The development of this high tension and of clonus was entirely unexpected and the speed of the tracing had to be altered twice during the course of this observation to obtain the latter part where the oscillations can be individually identified. Subsequent efforts to obtain better tracings were thwarted by the severity of the clonus and tension which became so great as almost to disrupt the apparatus.

In this particular case the initial tension is but little greater than the tension of "equilibrium." Why is it, if the irritability of the system in this case was greater than that previously observed, that the initial response was not proportionately greater than the "sustained tension?" We may suppose that each end-organ, or group of end-organs, is capable of stimulating a specific group of motor-units, beyond which lies a "fringe" out of range of these end-organs but to which they supply a subminimal stimulus, and beyond these again a group which they are unable to affect (3). Thus it becomes apparent that for each group of end-organs there is possible a maximal contraction which may fall far short of the maximal contraction for the muscle as a whole. If the irritability of the system were great

enough it is possible to visualize a state in which even slight stimulation of the end-organs might result in a maximal contraction for that group and that any greater stimulation of them would result in no further response. In such a case "equilibrium" would obviously be at a relatively high tension. In other words, as the irritability of the system increases, the tension of "equilibrium" gradually approaches the maximal contraction for that group of end organs. Thus it is possible that, in the observation cited here, the irritability of the system was so increased that the initial contraction was maximal, or nearly so, for the end-organs stimulated and that equilibrium was approaching near to maximal tension.

It should be made clear that this is a purely hypothetical explanation for which, in the experiment under consideration, there is no proof. Acceptance or dismissal of this explanation in no way alters the fundamental character of the hypothesis of "parallelism of the muscle-fibers and end-organs." It has been our purpose only to show that the phenomenon is readily explainable on the basis of this hypothesis.

However, our explanation leaves the development of clonus unexplained. Clinicians are well aware of the fact that clonus is present most frequently in those cases having the greatest spasticity. May these also have the greatest irritability of the reflex system?

It is also well known to the clinician that clonus is most readily elicited by a sudden stretch upon the muscle. This we may suppose produces a sudden synchronous volley of afferent impulses which in turn sets up a relatively synchronous efferent volley producing a strong muscular contraction, relaxing the stretch upon the end-organs, and thus terminating the afferent volley which is soon reproduced and the cycle continued so long as tension upon the muscle is maintained.

In cases of very severe rigidity (hyper-irritability of the reflex system?) the same effect may be produced by a less sudden stretch. Such are the conditions observed in our patient (fig. 7). The system was increased in irritability, because there occurred a far greater mechanical response to a given stretch than was commonly observed in the same system under other conditions, and the stretch was no more rapid (in fact not as rapid) as had been applied to the same system when less irritable (see fig. 3), yet clonus resulted. It is probable that in this highly irritable system a much smaller number of end-organs subjected to less stretch are capable of inducing a greater mechanical response and that, therefore, in spite of the greater tension developed by muscular contraction initially, "equilibrium" is developed at a relatively higher level than is seen in other observations on less irritable preparations. Nevertheless this contraction must to some extent relax the pull upon the end-organs, but as fewer end-organs under less tension are required to produce "equilibrium" the major part of the initial tension is maintained. However, those end-organs which are in

this manner slackened or "protected" result in the relaxation of a few motor units, probably those nearest the subminimal fringe, and we see an alternating contraction and relaxation of those few fibers on the great mass of tension, like waves dancing upon a sea, for it will be observed that the oscillations which constitute the clonus are of slight intensity as compared with the basic tension.

The question must be raised at once as to why, therefore, clonus at "equilibrium" is not observed in all cases. The answer is that in all probability such oscillations do occur in many cases but that the threshold of the system is so high that the rhythmic mechanical response is too slight to be apparent. But in cases of increased irritability the sphere of influence, *i.e.*, the motor field, of a small group of end-organs is so increased that whereas ordinarily those end-organs would have activated very few motor units, not sufficient to produce alterations in tension perceptible by crude methods, they now activate a larger number of units which respond to a greater degree and produce movement known as clonus.

It was shown some time ago that the development of clonus is dependent upon synchronization of the efferent impulses to the muscle, as compared with complete asynchronization commonly seen in decerebrate rigidity (Denny-Brown (2)). It may still be asked, why are the efferent volleys synchronous and why are they rhythmical in clonus as compared with ordinary decerebrate rigidity in which there is also a constant relaxing and tensing of the end-organs which is asynchronous. It seems common agreement that all end-organs are not equally susceptible to stretch, and that, therefore, whereas a given stretch will pull upon certain end-organs it will not affect others and thus as the stretching progresses other end-organs are stimulated later than the first and irregularity of afferent flow develops. (This is, of course, not all of the situation for as stretch increases tension upon these end-organs first stimulated becomes greater than upon those stimulated later and an alteration in frequency of response also occurs.) In addition, the motor field of the group of end-organs first stimulated may lie completely outside the motor field of the second or they may overlap slightly so that not only is the afferent flow irregular but the efferent discharge is also irregular. While in a hyperirritable preparation the motor field of a given group of end-organs is so increased that it may largely or completely overlap the field of the second group (thus the effect of the initial afferent volley from the second group falls upon sterile ground, *i.e.*, motor units already in activity), with the contraction of these motor units not only is the first group of end-organs relaxed (and thus "protected") but the second group as well, and upon the subsidence of muscular contraction both groups become stretched simultaneously, though not equally, becoming thus synchronous in their response and drawing into the motor field of the combined groups of end-organs those few motor units, if any, which

were not included in the overlap of the motor field of the first group upon that of the second.

Thus one would anticipate a clonus to be of ever increasing intensity and we believe that at times that is observed to occur, within limits. However, when the irritability is so great that "equilibrium" approximates maximal response for the end-organs concerned, the possibilities for increase are slight. And it must be further recalled that the maximal response for the end-organs concerned during the "equilibrium" is less than for those concerned during the initial response to progressive stretch for at that time contractile tension in the muscle being less more end-organs are "exposed" and therefore more are concerned. Those which are completely "protected" by muscular contraction during "equilibrium" are not susceptible to stretch and are therefore no more concerned than those beyond the limits of possibility for the existing degree of stretch to stimulate and which were never involved in the response at all.

In concluding we wish to state that we do not consider that either the theory of "autogenous inhibition" or of the "subsidence of afferent flow" as explained by "parallelism" of the muscle-fibers and the end-organs as cause of the "lengthening reaction" has been proved. Not until careful studies of the electrical potential in afferent nerves leading from the muscles concerned have been made and the presence of a volley of impulses been demonstrated immediately preceding relaxation, to coincide with the "silent period" observed in the action current of the muscle during the knee-jerk, and which may therefore be reasonably thought to be inhibitory in function, can the theory of "autogenous inhibition" be considered as supported by factual evidence. The hypothesis here advanced has a little more factual evidence for its support. Matthews has conclusively proved that artificially induced contraction in the muscle silences the firing end-organ which is responding to passive stretch. But his demonstration must be used with caution as applying to the "lengthening reaction." The investigation was conducted with the muscle of a frog. It needs, therefore, to be confirmed for mammals, especially those highest in the series such as man and monkey as well as for the dog and the cat and this has in some measure been achieved by Forbes, Campbell, and Williams (4). Moreover the subsidence of afferent impulses was in response to mechanical contraction artificially induced. This should not matter, but that it really does not is susceptible of proof and should be submitted to further investigation. And third, Matthew's experiment (9) can in no way be interpreted as an operation of the "lengthening reaction." It was not intended to be. So like the first theory this too must be submitted to careful examination and it must be demonstrated that there is an arrest of afferent impulses secondary to the contraction in such a chronological relation as adequately to explain the relaxation and the subsidence of efferent impulses.

SUMMARY

1. The theory of "autogenous inhibition" as an explanation of the "lengthening reaction" rests upon too little evidence to warrant the wide acceptance which it is accorded, although tension which actually endangers the integrity of the muscle and its attachment *may* produce inhibitory afferent impulses capable of protecting the muscle.

2. A theory which we have termed the "subsidence of afferent flow," originally conceived by Fulton and Pi-Suñer, and further greatly supported by the work of Matthews, and Forbes, Campbell and Williams, explains the cessation of action currents during active contraction of the muscle.

3. This theory will not only adequately account for the "lengthening reaction" but also explains many of the phenomena seen during the muscular response to the stretch reflex in human skeletal muscle.

4. It has the advantage over the theory of "autogenous inhibition" that it does not require the addition of further theoretical mechanisms for its explanation.

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ON THE SYMPATHETIC INNERVATION OF GUINEA-PIG INTESTINE[1]

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A recent publication by Bernheim and Blockson (1932), purporting to show an alteration of the action of epinephrine on the intestine of the guinea pig, seems to call for the report of observations made in this laboratory during the past few years. The authors of the above mentioned article used ordinary Magnus preparations of excised guinea-pig intestine and found that when epinephrine was applied to the intestine during a pilocarpine contraction, there was a further contraction, which in most instances was followed by some degree of relaxation. Epinephrine applied during histamine contraction of the intestine caused a simple relaxation. Epinephrine alone resulted in simple relaxation. Bernheim and Blockson (1932) used mainly segments of ileum, but they report that in general the responses of the duodenum were similar to those of the ileum.

The results to be reported from our laboratory were of experiments with three types of preparation of excised intestine of the guinea pig, immersed in Sollmann-Rademaekers solution.

A. *Simple Magnus preparations.* Strips of guinea-pig duodenum responded to epinephrine by either simple contraction, or by contraction followed by relaxation. Strips of lower ileum usually showed a simple inhibitory response, although a preliminary contraction was occasionally observed.

B. *Excised "muscle-nerve" preparations,* previously described for rabbit and dog intestine (Hendricks and Thienes, 1931). Most of the guinea-pig preparations were taken from the middle or upper ileum. Electrical stimulation of the perivascular mesenteric nerves resulted in a preliminary contraction, followed by inhibition. Application of epinephrine to such segments upon recovery from nerve stimulation, duplicated almost perfectly the effects of stimulation of the nerve. Ergotamine[2] abolished the response to nerve stimulation.

[1] Supported in part by grants 194 and 236 of the Committee on Scientific Research of the American Medical Association.

[2] The ergotamine methanesulphonate was generously supplied by Sandoz Chemical Works, Inc.

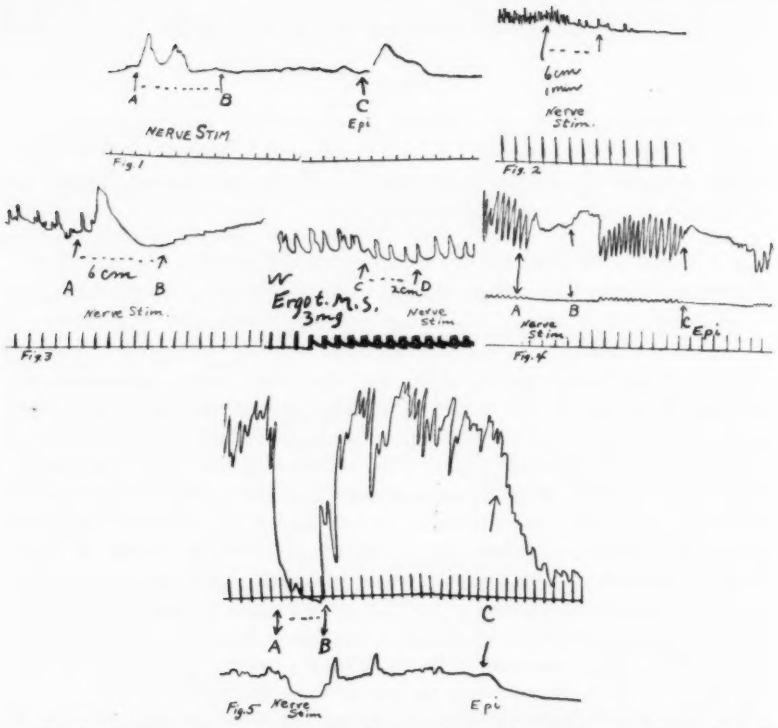

Fig. 1. Quiescent excised segment of upper ileum of guinea pig. From *A* to *B*, faradic stimulation of perivascular mesenteric nerves with secondary coil of Harvard inductorium at 7 cm. At *C*, epinephrine, 1:1,000,000. Between *B* and *C*, ten-minute interval. Time markings, 15 seconds.

Fig. 2. Active excised segment of lower ileum of guinea pig. Between arrows, faradic stimulation of perivascular mesenteric nerves, secondary coil at 6 cm. Time markings, 15 seconds.

Fig. 3. Active excised segment of middle ileum of guinea pig. From *A* to *B*, faradic stimulation of perivascular mesenteric nerves, secondary coil at 6 cm. caused contraction, followed by inhibition. At *W*, 50 cc. fresh Sollmann-Rademaeker solution and 3 mgm. ergotamine methanesulphonate; tracing stopped for ten minutes after the addition of ergotamine. From *C* to *D*, faradic stimulation of perivascular mesenteric nerves, secondary coil at 2 cm., no longer altered the activity of the muscle, due to paralysis of the neuromuscular connection by ergotamine. Time markings, 15 seconds.

Fig. 4. Modified Trendelenburg preparation of guinea pig ileum. From above downward longitudinal muscle, circular muscle, time record. Pressure within intestinal lumen, 2 cm. water. From *A* to *B*, faradic stimulation of perivascular mesenteric nerves, secondary coil at 12 cm.; at *C*, epinephrine, 1:1,000,000. Time markings, 15 seconds.

Fig. 5. Modified Trendelenburg preparation of guinea-pig colon. From above downward, longitudional muscle, time record, circular muscle. Pressure in intestinal lumen, 1 cm. water. From *A* to *B*, faradic stimulation of perivascular mesenteric nerves, secondary coil at 3 cm.; at *C*, epinephrine, 1:1,000,000. About ten-minute interval between *B* and *C*. Time markings, 15 seconds.

C. *Modified Trendelenburg preparations.* A previous report (Hockett, 1929) may be consulted for the details of this method. By this method are recorded both the activity of the longitudinal muscle and the volume of the lumen of the segment. Since the volume of the lumen has been shown largely dependent upon the state of the circular muscle, the record of the volume changes is interpreted as a record of the activity of circular muscle. Pressure within the lumen is maintained at a constant level by means of a large reservoir.

Stimulation of the perivascular mesenteric nerves as well as application of epinephrine resulted in contraction of the longitudional muscle, followed, in about two-thirds of the trials, by relaxation. In these experiments the circular muscle was relaxed and inhibited as a primary effect of either nerve stimulation or epinephrine.

Figures 1 to 5 illustrate the above described phenomena.

COMMENT. It is quite apparent that no special "treatment" of the guinea-pig intestine (as with pilocarpine) is necessary to cause it to respond to sympathetic stimulation with contraction. Our observations show that at the two extremes of the small intestine, opposite effects of sympathetic stimulation may be expected; contraction of the first part of the duodenum, relaxation of the terminal ileum (and colon, as shown by a few tests). The middle portion of the small intestine is first contracted, then relaxed by epinephrine or nerve stimulation.

These experiments add support to observations on other species in this laboratory (Hendricks and Thienes, 1931) that stimulation of the perivascular mesenteric nerves of excised small intestine, immersed in balanced salt solution, simulated closely, if not exactly, the action of epinephrine. Three possible explanations of this "unitary" effect of mesenteric nerve stimulation may be suggested.

1. The vagus effect is overshadowed by the sympathetic. Against this is the fact that ergotamine prevents entirely the effect of nerve stimulation. If the vagus nerves were functioning, but hidden by the sympathetic effect, paralysis of the sympathetic "endings" by ergotamine should allow the vagus action to become manifest.

2. Vagal action is not manifest because the vagus fibers terminate in the ganglia of the intestinal wall, and these rapidly lose their function on removal of their blood supply or central connections. There is some evidence to support this view, but it is still in the realm of the hypothetical.

3. There are no vagus fibers near the major vascular structures of the mesentery. Evidence for and against this statement could be cited, but discussion will be reserved until work on the point is completed in this laboratory.

SUMMARY

Stimulation of the sympathetic nerves to the cephalic half of the guinea-pig intestine, by faradization or by epinephrine, resulted in contraction of the longitudinal muscle, followed by relaxation. The circular muscle was relaxed and inhibited. In the caudal half of the guinea-pig intestine, the response to sympathetic stimulation was less augmentor in character, the inhibitory response predominating.

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MULTIPLE OVARIES IN MICE

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We have repeated the experiments of Friedman and Nice (1930) concerned with the effect of multiple ovaries on the oestrous cycle, using mice instead of rats and altering the transplantation as follows: instead of transplanting one ovary onto the musculature of the back and one onto the kidney, we have used two series of animals, one receiving two ovaries on a kidney, another receiving the two ovaries on the back symmetrically placed at a site on the dorsal musculature favorable for successful transplantation. Mortality in the series of animals receiving extra ovaries on the kidneys was rather high, but the nine animals of this series gave results identical with those had with our more extensive group receiving extra ovaries in the back. All the females used were albinos of the inbred Bagg-Little strain. All females used were virgin. Vaginal smears were taken daily beginning at the age when animals of this strain normally have their first litters. All females used as hosts had had six regular oestrous cycles, as indicated by the vaginal smear, before transplantation was made. The transplantations were made at various times during the cycle from pro-oestrus to dioestrus; our data show no correlation between the time during the cycle when transplantation is made and the subsequent oestrous history; neither is the oestrous state of the donor correlated with the subsequent oestrous history of the transplant host.

We have never obtained the results described by Friedman and Nice (1931). Their data show a halving of the duration of the oestrous cycle as indicated by the vaginal smear. In our operated animals the length of the vaginal cycle either remained unchanged after the operation or increased, in some cases exhibiting a marked inhibition of oestrus. Our data are summarized in table 1. The control set of transplantations listed in this table consisted of transplants of muscle tissue of the same size as the ovarian grafts.

The only significant change in cycle length is observed in the cycle immediately following transplantation in animals receiving extra ovaries. The statistical comparisons are made in table 2.

The inference is that animals receiving extra ovaries show, on the aver-

age, a significant inhibition of oestrus during the first cycle following transplantation, but that thereafter they exhibit cycles of normal length. Our first thought was that oestrous inhibition occurred as long as a successful "take" of the transplant was had, and that the inhibition disappeared with the resorption of the graft. The examination of a series of 43 animals who had received extra ovaries revealed the fact that the grafts persisted in about 65 *per cent* of the animals for an appreciable period (ordinarily for at least six weeks). A significant lengthening of the first post-operative cycle occurred only in about 45 *per cent* of our operated animals, how-

TABLE 1

The length of the oestrous cycle, as indicated by vaginal smears, in female mice before and after receiving certain grafts

TYPE OF TRANSPLANT	NUMBER OF ANIMALS OPERATED	AVERAGE LENGTH OF CYCLES BEFORE OPERATION, DAYS						AVERAGE LENGTH OF CYCLES AFTER OPERATION, DAYS					
Ovaries	48	6.98 ±0.23	7.42 ±0.25	7.00 ±0.23	6.79 ±0.20	6.69 ±0.22	7.04 ±0.25	14.35 ±1.00	8.06 ±0.30	7.20 ±0.42	7.80 ±0.50	6.81 ±0.39	6.00 ±0.33
Muscle	11	7.72 ±0.51	6.45 ±0.44	7.00 ±0.68	7.09 ±0.36	6.27 ±0.27	7.81 ±0.63	8.63 ±0.39	8.36 ±0.78	7.50 ±0.68	7.10 ±1.07	8.50 ±0.31	8.00 ±0.66

TABLE 2

The comparison of cycle lengths in several groups of animals (cf. *table 1*)

CYCLE	AVERAGE LENGTH, DAYS	DIFFERENCE, DAYS	PROBABLE ERROR OF THE DIFFERENCE, DAYS	DIFFERENCE / PROBABLE ERROR
Just before operation (ovary grafts)	7.04	7.31	±1.03	7.10*
Just after operation (ovary grafts)	14.35			
Just before operation (muscle grafts)	7.81	0.82	±0.73	1.12
Just after operation (muscle grafts)	8.63			
Just after operation (ovary grafts)	14.35	5.72	±1.07	5.35*
Just after operation (muscle grafts)	8.63			

* The difference is statistically significant.

ever. But apparently healthy transplanted ovarian tissue was found both in animals who had shown a lengthened post-operative cycle and in animals whose cycles were of normal length. The presence or absence of transplanted tissue was not, therefore, the cause *per se* of the observed inhibition. Furthermore, the muscle transplants in our control series persisted in all animals receiving them.

Nor does the distribution of cycle lengths in the critical post-operative period give any hint as to the nature of the presumed inhibition. The cycle lengths in the pre-operative cycles form a fairly symmetrical normal dis-

tribution curve with the mode at about seven days, with extremes at three and thirteen days. The data for the first cycle after operation form a definite skewed curve with the mode again at seven days and extremes at 3 and 73 days. The presence of a mode at seven days means merely that a good proportion of the animals operated upon showed no significant oestrous inhibition.

In a few cases we have available histological preparations of transplanted and normal ovarian tissue taken from animals definitely in a state of oestrous inhibition. Both the transplanted and normal ovaries are remarkably free of luteinized tissue. This is not true of ovarian tissue in animals with normal cycle lengths: the normal ovaries contain several sets of corpora lutea, although the transplanted tissue may not be markedly luteinized. We do not wish to stress these histological findings, however, since the data are so scant, and believe it sufficient to state that any explanation of the post-operative inhibition of oestrus depends on the functional state of the transplanted tissue which may or may not be reflected by the histological picture.

SUMMARY

Female mice receiving extra ovaries show either no change in the length of the oestrous (vaginal) cycle after transplantation or a significant lengthening of the first cycle after operation, with normal cycle lengths thereafter. The comparison of the data assembled from these animals and a group of control animals who received muscle tissue transplants indicates that the post-operative inhibition is statistically significant. No correlation was found between the apparent successful taking of the ovary transplants and the lengthened post-operative cycle.

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THE EFFECT OF ABDOMINAL DISTENTION AND RELEASE ON THE BLOOD PRESSURES IN THE ARTERIES AND VEINS[1]

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There is considerable evidence that variations in intra-abdominal pressure may produce significant effects on the cardiovascular system. Syncope and death have been reported to follow the removal of ascitic fluid or the abrupt release of other forms of excessive abdominal distention. Plumier (1909) found that the pressure in the inferior vena cava rose and then gradually subsided during abdominal distention in dogs. The pressure in the superior vena cava showed comparatively little change. Similar results were obtained by Thorington and Schmidt (1923). Frey (1923) found that compression of the abdomen of rabbits and dogs caused a consistent rise in arterial pressure, a slight elevation in jugular pressure and an increase in pressure in the femoral vein. Section of the depressor nerves, both vagi and sympathetics, failed to modify the result, suggesting that the blood pressure changes were due to mechanical rather than reflex action. Runge (1924) observed that the venous pressure in the lower extremities of pregnant women was higher than in the cubital vein, a difference disappearing after delivery. Villaret and Saint-Girons (1924) found the venous pressure in the lower extremities to be higher than in the cubital vein in ascites.

Our experiments were planned to determine the changes in venous and arterial blood pressure caused 1, by distention of the abdomen lasting a few seconds, a few minutes (up to 30), and several days, and 2, by release of such abdominal distentions. (The various periods of distention will be referred to in this report as acute, prolonged, and chronic, respectively.) It was hoped that information might be obtained on the possible mechanism of syncope or death which sometimes follows removal of ascites. The effects on respiration and heart volume were studied with the same end in view. Care was taken to maintain the intra-abdominal distention at a uniform

[1] Aided by the Emil and Fanny Wedeles Fund of the Michael Reese Hospital for the Study of Diseases of the Heart and Circulation.

level until ready to release it, in order to simulate clinical conditions more closely.

METHOD. Dogs were used, under morphine-sodium barbital anesthesia. The abdomen in the acute and prolonged experiments was distended with air, either free or in a balloon within the abdominal cavity, by means of a glass cannula and rubber bulb. Distention in the chronic experiments was maintained for three to seven days by an initial injection of a liter of mineral oil intraperitoneally and thereafter by injection of 200 to 300 cc. of oil daily to maintain the distention. Release of distention in the acute and prolonged experiments was abrupt. In the chronic experiments the oil was rapidly released by a wide incision in the lateral wall of the abdomen.

Venous pressure was measured with saline manometers by means of a catheter inserted into the superior vena cava via the external jugular vein, and into the iliac vein via the femoral vein. The mean arterial blood pressure of the carotid artery was measured in the usual way. Respiratory pressure changes of the intrapleural cavity were recorded with a tambour. The heart volume was measured with a glass oncometer using a segment capsule type of tambour, according to the technique of Wiggers and Katz (1922).

RESULTS. *Distention.* Rapid abdominal distention, performed 48 times, caused in all instances a prompt rise of pressure in the iliac vein of from 8 to 62 cm. of saline. The rise in venous pressure varied with the degree of abdominal distention and was maintained until the distention was released. At the same time, the pressure in the superior vena cava rose slightly or remained unchanged. The pressure in the superior and inferior vena cava varied in a similar manner in six prolonged experiments except in one instance of extreme distention where the rise in superior vena cava pressure attained a height of 7 cm. of saline. These results correspond with those obtained by Plumier (1909). Sectioning of the vagi, in four further experiments, did not alter the effects of the distention. The pressure in the inferior vena cava in the chronic experiments was well above the usual normal while that in the superior vena cava was within normal limits.

Variable arterial blood pressure changes were observed on distention in 45 acute experiments. A fall in arterial blood pressure was noted in 27 and a rise in 18 trials. These changes did not persist in all cases during distention. The initial fall in arterial pressure was maintained in only 16 instances, returning to nearly the normal level in 10, and attaining a higher level in one. The initial rise, however, was maintained above the normal level in 17 experiments and fell subsequently in only one. Prolonged distention of marked degree caused a slowly progressive fall in carotid arterial pressure to a level well below the previous "normal."

The stroke volume of the heart was increased in all but one of 15 experi-

ments while the abdomen was being rapidly distended. This was followed in some instances by a slight decrease during abdominal distention to a level slightly below or above the previous "normal." In most instances, however, the initial increase in stroke volume was maintained throughout the period of distention. Care was taken in all experiments to minimize the effect of the diaphragmatic excursions on the oncometer.

Release of distention. Abrupt release of abdominal distention resulted in a prompt return of the pressure in the inferior vena cava toward its previous level. In 21 experiments only was this return exactly to its previous level; in eight the venous pressure remained from 0.5 to 2.0 cm. above, and in 10 experiments from 0.5 to 2.5 cm. below the original level. The pressure in the superior vena cava showed only slight variations on release of distention, although in some of the prolonged experiments it fell slightly below its pre-existing level. Similar results were obtained in four further experiments in which the vagi were previously sectioned. The pressure in the inferior vena cava in the three chronic experiments fell promptly to "normal" levels after rapid removal of the oil. The pressure in the superior vena cava also showed a moderate fall on release of distention, the average being 1.5 to 2.0 cm. of saline.

Release of abdominal distention in the acute experiments resulted in a fall in the arterial pressure below the "normal" level in 22 experiments. In one experiment the blood pressure remained below the normal; in 19 it rapidly rose to nearly the "normal" level and in two it rose to a level above normal. In 13 experiments an initial rise in arterial blood pressure occurred on release of distention and in one experiment no change was apparent. In nine of the former experiments the elevation in arterial pressure was maintained, in one it continued to rise for some time and in three it fell again to below normal. It is noteworthy that the blood pressure changes were not always of the same degree nor did they always occur in the same direction when the experiment was repeated several times in the same animal. These variations did not depend on the apparent condition of the heart, nor could they be correlated with the changes during distention. In two animals section of both vagi greatly exaggerated the extent and abruptness of the arterial blood pressure change. The arterial blood pressure in the prolonged experiments also showed variable changes on release of abdominal distention. In some there was an immediate rapid rise, in others this rise was preceded by a further fall of short duration. The remainder showed a fall which often persisted below the control level (cf. fig. 1).

Abrupt release sometimes caused an increase but more often a decrease in stroke volume. In one experiment practically no change occurred. In several experiments the decrease in stroke volume was transient (cf. fig. 1).

Discussion. Changes in depth and rate of respirations were observed during abdominal distention and release in the few experiments studied.

While the changes in respirations could at times be correlated with the blood pressure variations, other experiments showed no correlation. Furthermore, the use of artificial respiration at a constant rate did not nullify the effects of distention and release, showing that respiration was not the prime factor in the type of circulatory change observed.

The rise in pressure in the inferior vena cava upon abdominal distention can be explained by the blocking of this vessel cephalad to the point where the pressure was measured, with the consequent accumulation of blood behind the block. The fact that the elevated pressure in these veins was maintained even in prolonged distention supports the view that no appreciable change in the bed behind the obstruction occurred, or that the increase in bed that did occur was forced by the pressure of the accumulat-

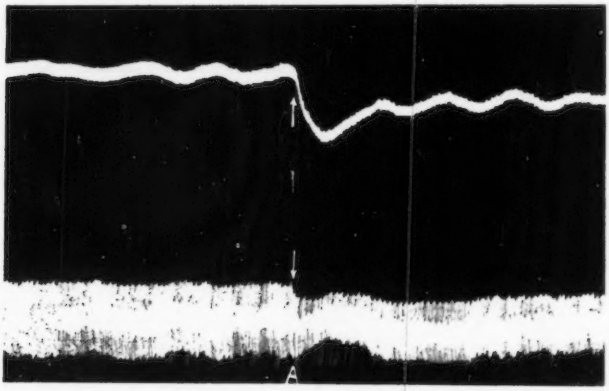

Fig. 1. Effect of release of abdominal distention at *A* on the mean carotid arterial blood pressure (upper curve) and cardiac volume (lower curve).

ing blood. The slight progressive rise in pressure in the superior vena cava occasionally observed cannot be explained easily. The prompt return of pressure in the inferior vena cava towards normal after abrupt release of distention is a reflection of the rapid accommodation which is possible when the intra-abdominal pressure is reduced. In accordance with Frey (1923) we found that the vagus nerves were of little or no importance in the behavior of the venous pressure.

The changes in arterial blood pressure observed in our experiments differ from those reported by Plumier (1909) and by Frey (1923) who state that the carotid pressure rose during distention of the abdomen, and are more in line with the results reported by Thorington and Schmidt (1923) who obtained variable results in four animal experiments. The drop in arterial blood pressure in three-fourths of our experiments on abrupt distention of

the abdomen contrasted with the rise noted in the remaining instances. These variable blood pressure results are due to several opposing factors. The abrupt occlusion of the inferior vena cava by preventing the return of blood to the heart causes an abrupt reduction in the discharge from the heart into the aorta. Marked upward displacement of the diaphragm and slower breathing may further limit the output of the heart. The kinking action of the tense and elevated diaphragm on the vena cava may also play a rôle. The decrease in the amplitude of the pressure oscillations, found in some cases when the blood pressure fell during marked abdominal distention, is in favor of this view, as is the transient decrease in stroke volume sometimes recorded.

These factors tending to depress arterial blood pressure are counteracted by other changes which tend to elevate the carotid pressure. Rapid compression of the inferior vena cava might squeeze some blood into the heart at a rate more rapid than normal. This could temporarily raise arterial blood pressure by the transient increase of blood flow to the heart. Another factor tending to elevate arterial blood pressure would be the increase in peripheral resistance caused by a variable degree of compression of the abdominal aorta during distention. Such an interplay of opposing factors could explain at least in great part the variable changes in arterial pressure observed during abdominal distention.

The interplay of opposing factors, the reverse of those previously mentioned, can account for the variable changes in blood pressure on release of abdominal distention. Important is the observation that the fall in blood pressure on release of abdominal distention may be as much as 40 mm. of mercury and that this low level may be sustained for some time. The clinical significance of the frequent fall in blood pressure during release of distention is obvious and may be an important factor in the production of syncope occasionally observed on release of ascites or other forms of clinical distention. A temporary fall in arterial pressure may lead only to syncope, but a sustained drop, if of sufficient degree to reach shock level in a feeble patient, might result in death.

The factors involved in such a fall in arterial blood pressure on release of distention may arise either in the venous, capillary or arterial systems. It is conceivable that the intra-abdominal veins, compressed during abdominal distention, may act as a reservoir for the blood otherwise returning to the heart when distention is abruptly released. Blood from other regions might flow into these comparatively empty veins thus reducing the return to the heart with a consequent fall in arterial pressure. Such an event should be reflected in a fall below normal of the pressure in the superior or inferior vena cava. But the pressure changes in the superior vena cava were very slight, occurred in only a few instances and were not observed to fall below the control level. The pressure in the iliac vein did at times reach

a level slightly below normal but this occurred several seconds after abdominal distention was released and definitely after the blood pressure had fallen. Furthermore, since little or no blood was returned to the heart by the inferior vena cava during distention further reduction via this channel could hardly occur when distention was released.

A much simpler explanation can be found in the changes in the arterial system.[2] Rapid release of abdominal distention quickly reduces the peripheral resistance by abruptly releasing the partially compressed abdominal aorta.[3] This will result in a quick and marked fall in arterial pressure, before the unequally distributed blood in the venous system returns to a normal balance. Simultaneous readings of pressure in the carotid artery and the venae cavae showed that the arterial pressure actually fell before the venous. The results of such a sharp and abrupt fall in arterial pressure must affect the coronary flow and cardiac output adversely (cf. fig. 1), and such a drop in pressure may produce cerebral anemia. Either or both of these factors can result in syncope and even death.

The importance of changes in the venous and capillary systems in explaining the effects of abdominal pressure release has been overestimated. More reliance should be placed on measures tending to raise the arterial pressure quickly. Adrenalin and similar agents should prove of value in the immediate treatment of such emergencies.

SUMMARY

1. The effects of induction and release of abdominal distention on venous pressure, arterial pressure, and cardiac stroke volume were studied in a series of 51 experiments.

2. The pressure in the inferior vena cava rose when the abdomen was distended while the pressure in the superior vena cava was slightly elevated or but little affected. Release of distention resulted in a prompt return of pressure in the inferior vena cava to normal. These results were approximately the same in experiments of short and long duration and in instances where distention was maintained for several days.

3. Arterial pressure fell in 27 of 48 experiments during distention of the abdomen and rose in 21 others. In 34 experiments a fall to a level below the normal occurred on abrupt release of abdominal distention. This was mirrored in the changes in stroke volume. The interplay of various factors responsible for these changes is discussed.

4. On release of distention of the abdomen a fall of arterial blood pressure

[2] The changes in arterial pressure during distention and release are probably modified by pressor and depressor reflexes set up via the splanchnics.

[3] And partially compressed intra-abdominal arterioles and capillaries.

was usually obtained. The fall in arterial pressure was 40 mm. of mercury in some instances and was sustained at that low level in a few.

5. The clinical importance of such a fall in arterial pressure on release of distention as a cause of syncope and death is discussed and adrenalin suggested as a rational form of treatment in the emergency.

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HEAT REGULATION AND WATER EXCHANGE

XVI. Anhydremia with Liver Hydration of Neurogenic Origin

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For many years it has been appreciated not only that body temperature is subject to cerebral control, but also that certain operative procedures confined to the cerebrum are capable of inducing a febrile rise of temperature. Furthermore it is also well known that the onset of fever is usually associated with blood concentration. The fate of the water lost from the blood at this time has been traced by Barbour and Marshall (1931) in several varieties of toxic fever, to the liver.

Hitherto neurogenic fevers do not appear to have been investigated in relation to the water exchange, although Barbour and Tolstoi (1924) showed that decerebration or cervical cord transection interferes with the blood concentration normally induced by cold baths in dogs. Rogers (1919) also has shown that pigeons, deprived of their basal ganglia, exhibit polyuria, being unable to retain water. Much work has been done relating both the pituitary and the hypothalamic region to the water exchange (Underhill, 1932).

The first object of the present work was to study the effects of neurogenic fever, produced by either "heat puncture" or cooling of the basal ganglia, upon the blood concentration. In the event of the expected anhydremia, attention was then to be paid to the liver as a possible cause.

Influence of the brain on blood concentration. Normal, young adult rabbits were used, either males or non-pregnant females. They were kept upon a diet of hay, oats and potatoes with occasional carrots. Food but not water was removed the night before experimentation.

The brain operations were all made with the hollow U-shaped puncture tube described by one of us (1912). Light ether anesthesia was employed and stringent asepsis. The skull was entered by a 5 mm. trephine just to one side of the mid-line and just anterior to the coronal suture. After opening the dura bloodlessly, the holder for the puncture tube was screwed into the skull. A dressing of cotton and colloidin

[1] The experimental data are taken from the thesis of Benjamin F. Aydelotte, presented in candidacy for the degree of Master of Science, at the University of Louisville, 1931.

was used. In some cases the brain puncture was made at once, in other cases it was postponed until complete recovery from anesthesia. In either case the sudden introduction of the double tube through the cortex down to the basal ganglia and to the base of the skull rarely produced any immediate response on the part of the rabbit.

The blood concentration was followed by means of specific gravity determinations by the falling drop method. The blood for this purpose was taken from the marginal ear vein, the usual precautions being taken to prevent the slightest degree of excitement in the animal.

For influencing the temperature of the basal ganglia it was found convenient to keep hot and cold water respectively in five gallon jars well insulated by sawdust packing, from which a mixture of water kept constant at any desired temperature, could be led off. Contact of the rubber tube conveying the water, with any part of the rabbit's skin, was carefully avoided.

Effects of cooling and heating the basal ganglia. We shall now describe results from three experiments in which the brain puncture was made in the morning and the effects of temperature upon the basal ganglia were studied the same afternoon after recovery from ether.

In figure 1 will be seen the results on rectal temperature and blood specific gravity of the alternate application of heat and cold. It will be noted that the application of heat by running water at 47 to 48°C., through the tube for about 35 minutes resulted in a rapid fall in both rectal temperature and blood specific gravity. Polypnea was observed and the usual vasodilatation of the ears. After the heating had been discontinued for about twenty minutes, the blood specific gravity was found to be still lower although the temperature had returned above its original point. The application of cold at about eleven degrees was followed at once by vasoconstriction of the ears and a rapid rise in body temperature, "cold fever." The blood specific gravity, however, continued to fall for about ten minutes during which time the body temperature lost 0.3 of the 0.7 degree it had gained. From this time on, as the cold was continued, the blood specific gravity rose rapidly exhibiting a gain of 0.004 within the next thirty-eight minutes, while the body temperature remained constantly high. When the cold stimulus was removed, there was an immediate fall in specific gravity which, however, did not continue further under the application of heat at 43°C. for seventeen minutes although the temperature fall was typical. On again applying cold at about 18°, the body temperature at once began to recover and the specific gravity rapidly attained a new high level.

The experiment thus confirmed in general the expectation that cooling the basal ganglia would increase both temperature and specific gravity, while heating would have the opposite effect. The response of the blood, however, was sometimes slower than that of the body temperature, indicating that the temperature changes are due in part to other well known factors.

Another rabbit under similar circumstances, gave very prompt and characteristic responses to both cold and heat, twice, respectively. Some fatigue of the mechanisms involved appeared after about two hours of temperature application in that removal of a long continued heat stimulus (44°C.) was followed by a still further fall in body temperature although the

specific gravity response was characteristic. Subsequent application of cold gave the typical response in body temperature but a fall instead of rise in specific gravity. The classical effects of both the temperature procedures were constantly observed on both the ear vessels and the respiration, cold being the stimulus for vaso-constriction and slowing of the respiration, heat being followed by vasodilatation and polypnea. In a third experiment characteristic effects of both cold and heat on the temperature and blood specific gravity were noted. In this rabbit the very immediate fall in specific gravity on cessation of cold and its rise on cessation of heat, were especially well marked. In experiments 1 and 3 it was noted that temperatures of 43° and 41° respectively, gave less characteristic heating effects than did higher temperatures.

Effects of puncture fever. Although it was clear after the above experiments that the blood concentration is notably influenced in "cold fever" which is of neurogenic origin, it was decided to study the effects of simple puncture as well.

A rabbit was trephined under ether in the morning, the dura being split and the holding plug screwed into place. After complete recovery from ether, the puncture was made, as shown by the arrow in figure 2. There was a decrease in temperature during the first half-hour, the febrile rise not setting in until about two hours after the puncture. This delay in febrile response has often been observed after punctures made with this kind of tube. The blood specific gravity fell but slightly and for a brief time after the puncture and then a gradual rise set in which, however, became more abrupt as the body temperature went up. At the end of the figure is shown a slight remission in body temperature with a marked fall in blood concentration.

The results thus far described testify to the association of marked concentration of the blood with brain puncture fever and "cold fever" and to the contrary effects of heating the basal ganglia.

Blood concentration after liver denervation. In attacking the problem of the possible relations of anhydremia to the tissues in puncture fever and cold fever the possible rôle of the spleen was kept in mind, but our first procedure consisted in denervation of the liver. This denervation, according to the method of Plaut (1922) consisted in double ligation and section of the hepatic artery together with its plexus, in addition to which were cut all of the branches of the left vagus at the inferior end of the esophagus which do not lead directly to the stomach. Although the arterial section has the disadvantage of some interference with the oxygen supply to the liver, we found that the above procedure serves to bring to light the effects of denervation. Four rabbits in which the liver had been denervated were subsequently submitted to brain puncture. In two of these this puncture was done under ether and the effects of cooling the basal ganglia studied after recovery from ether. These four experiments were consistent in that *the striking changes of specific gravity which are normally produced in neuro-*

genic fever, were entirely absent. This would clearly relate the neurogenic anhydremia seen in the regulation against cold to the liver rather than to the spleen.

The following will serve to illustrate the effects of cold on the liver denervated rabbit:

The hepatic artery and plexus and the appropriate branches of the left vagus had been cut and the brain puncture made under ether, between 10:00 and 11:00 in the morning. The animal was apparently normal at noon, the zero hour in figure 3. He was, however, exhibiting a steady rise in body temperature which had not recovered from the effects of ether. At 1:30 p.m. a temperature of about 13°C. was applied to the basal ganglia. Shivering appeared, respiration was slowed and there was marked vasoconstriction in the ears. In spite of these changes, there was no significant

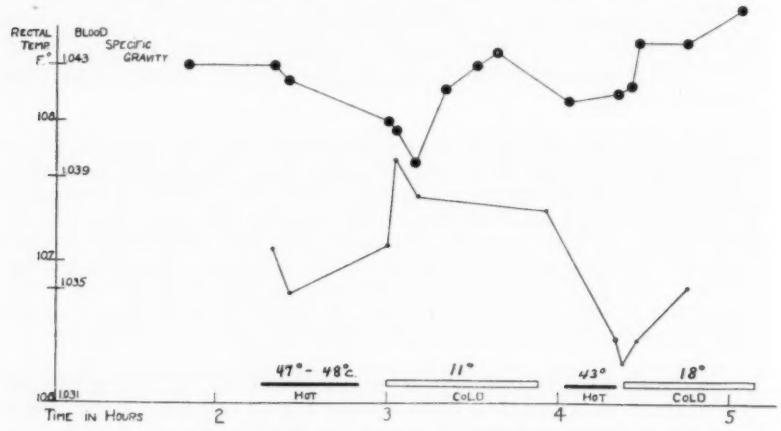

Fig. 1. White male rabbit 2600 grams. Fever puncture at 9:30 a.m. Abscissae: hours p.m.; ordinates: rectal temperature (degrees Fahrenheit) and blood specific gravity. Small dots, rectal temperature. Large dots, specific gravity. Solid lines, brain heating. Oblongs, brain cooling.

change at all in the specific gravity which remained almost constant throughout the experiment. The body temperature rose more slowly after the cold had been applied than before.

In another experiment denervation was performed together with the brain operation, without the puncture, which was deferred until the second day after. Figure 4 shows the body temperature and specific gravity after the original operation, and again on the next day. This was the day of the puncture, the time of which is indicated by the arrow. It will be noted that the specific gravity was maintained very constant before and after the puncture, at a level just below 1.045. Immediately following the puncture a marked rise in body temperature occurred. As the figure

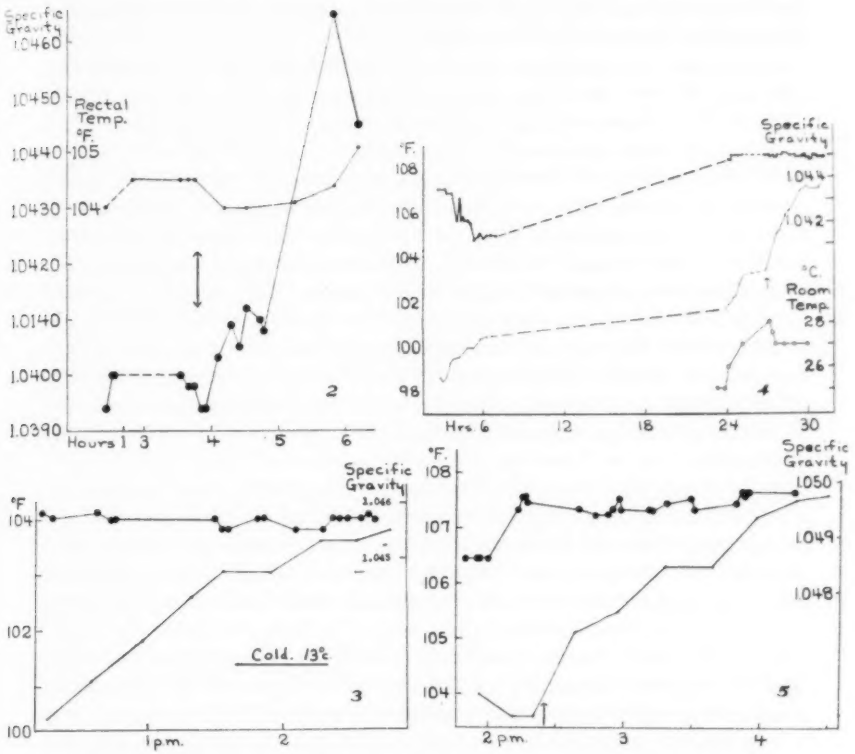

Fig. 2. Rabbit subjected to brain puncture. Abscissae: hours; ordinates: blood specific gravity and rectal temperature. Small dots, rectal temprature; large dots, blood specific gravity. Puncture of basal ganglia after recovery from anesthesia at time indicated by double arrow.

Fig. 3. Flemish male rabbit 2250 grams. Liver denervation at 10:15 a.m. Brain puncture at 11 a.m. Zero hour, noon. Abscissae: hours; ordinates: rectal temperature (degrees Fahrenheit) and blood specific gravity. Rectal temperature, small dots; specific gravity, large dots.

Fig. 4. Flemish female rabbit 4450 grams. 12–18–30: liver denervation and exposure of brain without puncture. 12–19–30: morning rectal temperature (small dots) and blood specific gravity (large dots) shown at left of figure (pulmonary râles were present). 12–20–30: brain puncture at 1:10 p.m. indicated by arrow (no pulmonary râles on this day). Abscissae: hours; ordinates: rectal temperature, blood specific gravity and room temperature (indicated by circles in right hand lower corner).

Fig. 5. New Zealand Red female rabbit 1765 grams. 10 a.m., liver denervation and exposure of brain without puncturing. 2:25 p.m., brain puncture shown by arrow. Abscissae: hours; ordinates: rectal temperature and blood specific gravity. Rectal temperature, small dots; blood specific gravity, large dots.

shows, this was independent of the room temperature which had influenced the morning temperature of the rabbit.

On another day puncture was done four and one-half hours after the operation of liver denervation and brain exposure. The resulting rise in temperature without change in specific gravity is shown in figure 5.

The above three experiments and another just as carefully performed in which cold applied to the basal ganglia was followed by a rise of 0.7°F. without any change whatever in the blood specific gravity, all lead very definitely to the conclusion that the mechanism for influencing the blood specific gravity through the basal ganglia was eliminated by denervation of the liver as performed in these experiments. The respective specific gravity increases in four normal rabbits were 0.0012, 0.0028, 0.0067 and 0.0065. With this may be contrasted the increases in three denervation experiments: 0.0005, 0.0002 and 0.0002. The rise in body temperature, which can still be induced by puncturing or cooling the basal ganglia, must therefore be ascribed primarily to the intact peripheral vasoconstricting mechanism.

The temperature increases were more consistently moderate in the absence than in the presence of the water shifting mechanism. In fact, in one case when the brain was cooled in the presence of a rising body temperature, the latter was depressed instead of showing an accelerated rise. The respective maximum increases in rectal temperature following puncture of the basal ganglia in four normal rabbits were 1.25°, 4.7°, 1.6° and 3.3°F.; three rabbits previously subjected to hepatic denervation showed respective rises of 2.0°, 2.9° and 2.5°F. Therefore the liver denervation did not alter the maximum temperature effect.

The water content of the liver in neurogenic fevers. To determine whether brain puncture or cooling of the basal ganglia exerts any influence upon the water content of the liver, the liver solids were determined on animals freshly killed, under the following conditions: normal, during puncture fever, during puncture fever combined with cooling the basal ganglia, during puncture fever induced after denervation of the liver, during puncture fever with heating of the basal ganglia, after liver denervation, puncture and cooling of the basal ganglia, after liver denervation and puncture and heating of the basal ganglia, and after hepatic denervation alone. In all we used twenty rabbits in three litters comprising five, seven and eight animals respectively—all young adults in healthy condition. They were killed by a blow at the base of the occiput and the liver removed almost immediately. Samples of one-half gram were obtained in every rabbit from each of the three large lobes, weighed on watch glasses and dried to a constant weight in an oven at about 105°C. As will appear below this afforded satisfactory triplicate determinations of the liver solids except after denervation of the liver.

In the first litter experiment the average liver solids were found to be 26.3 and 28.0 respectively in two normal rabbits. A rabbit of this litter, killed during puncture fever, showed a liver solids percentage of only 24.5; another one in which cooling the basal ganglia had been done for one-half hour beginning one and one-fourth hours after puncture, gave a liver solids percentage of 26.4. The last mentioned animal had exhibited a rise in blood specific gravity from 1.0432 to 1.0453 during the cooling. The evidence from these four rabbits favors the occurrence of liver hydration in neurogenic fevers.

Liver denervation was done in the fifth animal of the litter. This was followed by brain puncture from which a marked fever resulted, with vasoconstriction in the ears. The liver solids were found reduced to 21.5 per cent, showing the wettest liver of this litter. This degree of hydration has, however, also resulted from liver denervation alone.

In the second litter of rabbits the two normals gave liver solids percentages of 29.3 and 26.3 respectively, the average normal 27.8 per cent. Two rabbits treated by brain puncture only showed liver solids of 25.8 and 27.9 respectively. Here, as in the last litter, the most definite liver hydration was shown the shortest time after puncture. It is possible that a good deal of the excess water has been given up by the liver by the time one and three-quarters hours have elapsed, as in the second rabbit. Brain puncture after denervation of the liver gave the lowest liver solids of the series, in this case 24.2

It was necessary to delay the work on the other two rabbits for another week after which time liver denervation was performed, as usual by ligation and division of the hepatic artery and the animal allowed to recover entirely from the anesthesia. This rabbit was killed without further procedure and yielded a liver solids percentage of 21.9, confirming Afanassiew (1883) who showed that ligation of the hepatic artery under ether tends greatly to enlarge the liver cells. The last animal of the second litter was submitted to brain puncture and was killed three hours later, after half an hour of heating the basal ganglia. During this heating the rectal temperature fell 0.4°F., and the blood specific gravity dropped from 1.0531 to 1.0505. The resulting liver solids were 27.1 per cent, a figure nearer the average normal than the average puncture animals of this litter. It is probable that heating the brain tends to relieve the liver of some of its excess water, reversing the process resulting from cooling.

The final litter in which the liver solids were determined, consisted of eight rabbits. The two normal animals killed in this litter gave liver solids of 28.9 and 31.3 respectively, giving an average normal of 30.1 per cent. An animal submitted to heating the basal ganglia for thirty-five minutes, beginning two and one-half hours after puncture, showed a liver solids content of 27.8 per cent. Puncture followed by cooling the basal ganglia

under similar conditions, gave a liver solids of 26.6, indicating some liver hydration in both animals, which was much more marked in the case of the animal with the cooled brain. Two rabbits in this series were killed over five hours after simple puncture and yielded liver solids of 26.8 per cent and 28.1 per cent respectively (average 27.45 per cent).

In both of the two remaining rabbits of this litter, the liver was denervated and as usual very low liver solids resulted. These denervated rabbits were both subjected to brain puncture. In one of them cooling of the basal ganglia was followed by a liver solids percentage of 21.4, while in the other, after heating the same region, the liver solids amounted to 23.3 per cent. There is no question, therefore, that liver denervation as performed in this investigation, results in marked hydration of that organ.

One interesting observation emerges as a result of routinely examining the liver solids in all three lobes. While excellent agreement obtained throughout most of the experiments, a striking difference was seen in the right lobe of the liver in four of the five denervation experiments. This lobe was decidedly wetter than the other two lobes in the experiment where we performed liver denervation alone, also in one but not in the other rabbit where puncture alone was performed after denervation. In the two denervated rabbits submitted to puncture and the effects of temperature, a remarkable difference was noted. In the cooled rabbit the solids of the liver went down to 17.7 as against 23.1 and 23.5 respectively for the other two lobes; in the *heated* rabbit the right lobe liver solids exhibited practically a *normal* figure, 27.4 per cent as against 21.0 and 21.4 for the other two lobes respectively. These facts point strongly to the hypothesis that our operation in four out of five rabbits failed to destroy the innervation of the right lobe. Such an interpretation affords us three rabbits in which effects mediated by the basal ganglia upon one liver lobe are controlled in the same animal by negative results in denervated lobes. Thus puncture as well as cooling of the brain was able to cause the right lobe to take up a large amount of excess water, while heating the brain caused this lobe to give up all such extra water. Where liver denervation alone was performed the right lobe solids were 19.9 per cent against 22.8 per cent and 22.9 per cent in the other two lobes suggesting a selective denervation in this animal as well.

Emphasis upon this peculiarity of the right lobe in denervated rabbits is justified by the splendid agreement between the three lobes in fourteen out of fifteen non-denervated rabbits. The average of the mean deviations in these animals is ± 0.36 per cent of the liver solids.

Since all the experiments on the liver solids were performed on groups of young adult rabbits of the same stock, we have averaged the results from the same procedures in the different groups. The average of six normal rabbits was 28.3 per cent which corresponds closely to findings in many other livers in this laboratory.

To exclude etherization as a factor in our results with operated rabbits two further animals were killed shortly after recovering from ether narcosis. One of these rabbits had been given ether for thirty-three minutes, during which time his blood specific gravity rose from 1.0470 to 1.0494. One hour and twelve minutes after discontinuing the ether, it had fallen again to 1.0475, and as the animal appeared normal, he was deemed to have recovered from ether and killed. A second rabbit was etherized for thirty-five minutes, during which time the blood specific gravity rose from 1.0497 to 1.0538. One hour and twenty-seven minutes later it had fallen to 1.0498; the animal appeared normal and was killed. In each rabbit the triplicate liver solids determinations agreed closely. The average liver solids percentage in the first rabbit was 27.8, and in the second rabbit 28.3, giving a mean of 28.1. Thus we cannot regard etherization as a disturbing factor in the interpretation of our liver solids results.

Five puncture fever rabbits gave an average of 26.6 per cent liver solids, indicating hydration while the addition of cooling (in two further rabbits) the basal ganglia brought the average down to 26.4 per cent. Heating the same region in two rabbits brought the percentage level back to 27.4. Liver denervation in five rabbits, four of whom were treated further in various ways, yielded an average for the liver solids of 22.9 per cent.

Histology of the liver after brain puncture alone, with cooling and with heating. Samples of liver taken from animals in each type of procedure were preserved in formalin, embedded in parafin and stained with hematoxylin and eosin.

Microscopic examination of liver sections from rabbits killed during simple puncture fever gave very definite indications of edema. The central veins were found constricted and the sinusoids obliterated by the swelling. Under a magnification of 390, the Kupfer cells were seen to be compressed, the liver cells in general appearing hydropic, angular in outline and greatly enlarged. Numerous coarse granules were unevenly distributed throughout the clear hydropic cytoplasm. The nuclei were normally rounded and well stained. The hydropic condition was usually found more marked at the periphery of the lobule (plate 1).

The liver sections of rabbits killed during heating of the basal ganglia presented a picture not readily distinguishable from normal liver. The central veins were of good size and the sinusoids everywhere in their vicinity are prominent, giving the usual cord-like arrangement of the liver cells. Under a magnification of 390, the cell outlines were seen to be definitely free from angulation, the granules of the cytoplasm were small and compactly arranged with fairly uniform distribution, the nuclei very dark staining and of normal appearance (plate 2).

After the application of cold to the basal ganglia in puncture fever, the liver sections exhibited a similar hydropic swelling with even greater con-

striction of the central veins. The sinusoids appeared obliterated throughout the entire lobule with the exception of some small central areas. The Kupfer cells were elongated and flattened, the cytoplasmic particles being aggregated into relatively large granules. The nuclei appeared normal and stained well. No evidence of vascular congestion was seen. In some areas the cells are enormously enlarged presenting an angular outline (plate 3).

While the histological pictures give no conclusive evidence as to the relative amount of water contained in the organ under the different conditions, its distribution is very significant. It is clear from the first and third plates that the liver is in a marked state of edema during fever of neuro-

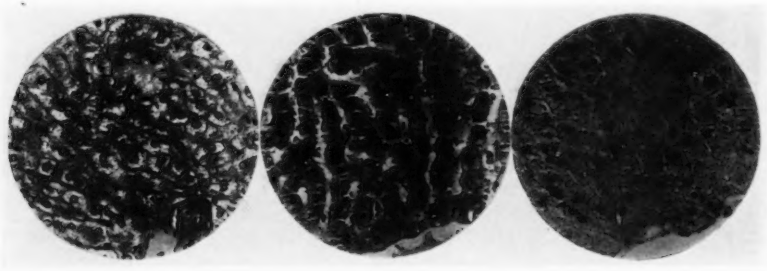

Plate 1 Plate 2 Plate 3

Plate 1. ×390. Liver from rabbit with "puncture fever." Central vein shown below.

Plate 2. ×390. Liver from rabbit with "puncture fever" reduced by heating brain. Central vein shown below.

Plate 3. ×390. Liver from rabbit with "puncture" and "cold fever." Central vein shown below.

genic origin, whether induced by simple brain puncture or brain puncture reinforced by cold. If we had no gravimetric evidence concerning the water we could not say that the third section was taken (as we know it was) from a liver containing relatively less water. It is abundantly evident, however, that a large per cent of the fluid is located outside rather than inside the hepatic cells, strongly suggesting, of course, that it is in process of being carried away by the lymphatics. The absence of vascular congestion in the neurogenic fevers affords conclusive evidence that the gain in fluid is not due to an increase in the blood content of the organ.

Histological study of the experimental livers therefore confirms the conclusions drawn from their respective water contents.

SUMMARY. We have shown that puncture of or cooling the basal ganglia of rabbits, besides the well known pyretic effects of "puncture fever,"

"cold fever," depressed respiration and vasoconstriction, causes anhydremia. The blood specific gravity often increases greatly; in two instances of puncture followed by cooling, this increase amounted respectively to 0.0065 and 0.0067. Conversely, heating the basal ganglia was found to reduce the blood specific gravity.

Liver denervation (method of Plaut) by section of the hepatic artery and its sheath, as well as the right esophageal branches of the vagus nerve, renders rabbits somewhat less susceptible to the pyrogenic respiratory and vasomotor effects of cooling the basal ganglia. This liver denervation entirely prevents the effects of cooling and heating the basal ganglia upon the blood concentration. The blood specific gravity remains exceedingly constant when the brain temperature is varied.

The above findings indicate that anhydremia is not an indispensable factor in fevers of neurogenic origin, but as shown by Marshall, Aydelotte and Barbour (1931), it evidently plays a marked rôle in retarding evaporation from the body surface in cocaine fever.

The denervation experiments having indicated the importance of the liver in the anhydremia in "puncture fever" and "cold fever," we next showed that these procedures are associated with liver hydration. The following average liver solids percentages of young adult rabbits were obtained in triplicate determinations:

6 normal rabbits 28.3 per cent
2 normal rabbits after ether 28.1 per cent
5 rabbits during "puncture fever" 26.6 per cent
2 "puncture fever" rabbits with cooled brains, 26.4 per cent
2 "puncture fever" rabbits with heated brains 27.4 per cent
5 rabbits with liver denervation (4 with additional procedures) 22.9 per cent

In four of the five liver denervation experiments there was evidence that the right lobe escaped for this lobe gave figures typical of normal responses to the different cerebral procedures,—contrasting strongly with the simple denervation effect shown by the other two lobes.

Histologically the livers of the "puncture fever" rabbits exhibited swollen cells with obliterated sinuses. The same evidences of hydration were seen after the brain had been cooled. The livers of rabbits with heated brains, on the other hand, appeared normal in character, with prominent veins and sinusoids and the usual cord-like arrangement of the liver cells.

CONCLUSIONS

1. The temperature of the brain influences the concentration of the blood of rabbits. Cooling raises and heat lowers the blood specific gravity. This control is lost by denervation of the liver.

2. Liver hydration occurs in the production of neurogenic fevers such as "puncture fever" and "cold fever."

3. Concentration of the blood is a factor favorable to but not essential to the production of neurogenic fever.

The authors are greatly indebted to Prof. A. J. Miller of the Department of Pathology, University of Louisville, for the microscopic preparations and to Prof. S. I. Kornhauser of the Department of Anatomy, University of Louisville, for the photomicrographs.

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METHYLENE BLUE AS AN ANTIDOTE TO CO POISONING

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These experiments are a continuation of those previously published on the effect of methylene blue as an antidote for carbon monoxide poisoning (1). In these experiments rabbits were tracheotomized and a cannula inserted which connected the trachea directly with the air containing CO. The expired air was separated from that inspired by means of a valve. The animals were not anesthetized. The experiments were done in two parts.

PART 1. Injections of methylene blue were made intravenously a few minutes before the animals received CO gas, and the time noted when respiration ceased as compared with the time required by those animals which did not receive the dye. CO was made by dropping formic acid in hot sulphuric acid and passing the gas over soda lime to take out any CO_2. In this set of experiments 10 per cent by volume of CO in air was used. The injections of methylene blue consisted of 1 cc. of 0.1 per cent methylene blue (Merck's medicinal) in Ringer's solution per kilo body weight. The dye was made up fresh for each set of experiments and warmed before injection.

Figure 1 shows the results. In this experiment 28 control animals and 26 treated were used. The variation curves show that the treated animals lived longer than the controls. The peak of those treated is at 6.5 minutes where 36 per cent stopped breathing as compared with 4.5 minutes in the controls where 39 per cent stopped breathing. In other words, in comparing these results, the treated animals lived 44 per cent longer than the controls.

PART 2. In this experiment the animals were allowed to breathe CO gas until respiration had stopped. After from 30 to 45 seconds, depending upon whether the cessation of respiration occurred before 6 minutes or not, injections of methylene blue were given intravenously and artificial respiration was begun by means of a respirometer. In this group there were 25 control animals and 20 treated. It was found that 20 per cent of the controls recovered, whereas 90 per cent of the treated animals recovered. The dye was effective only while circulation was still in progress. It required from 2 to 6 minutes for the animals to recover; in one case, 9 minutes.

These experiments confirm previous findings with rats (1). They show definitely that methylene blue is effective as an antidote for carbon monoxide poisoning given in conjunction with artificial respiration. They show further that methylene blue is effective in antagonizing the effects of CO if injected either before or after the animals were subjected to CO gas.

The theory upon which this work is based has been known a long time through the brilliant researches of Warburg (2). He showed that methylene blue antagonizes the effects of CO on the oxygen consumption of yeast cells. Later, Warburg, Kubowitz and Christian (3) found that the O uptake of red blood cells is practically unaffected by CO in the presence of enough methylene blue. Cook, Haldane and Mapson (4), using toluene-treated B. coli, found that the oxidation of succinate to fumarate, lactate to pyruvate and formate to bicarbonate by methylene blue is little affected by CO, if methylene blue is present, while the oxidations are inhibited by O_2

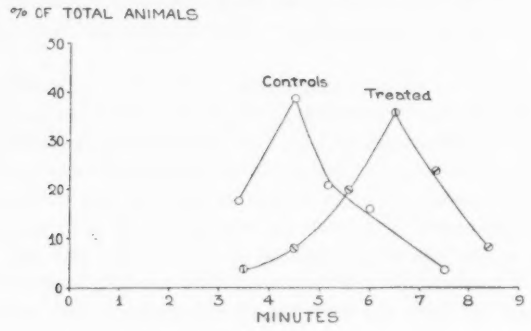

Fig. 1

in the absence of methylene blue. That methylene blue increases oxidations of animals has been known through the work of Heymans and Heymans (5), Barron (6), Harrop and Barron (7), and others. These previous investigators have furnished the background upon which the experiments herewith reported have been done.

While the mechanism (8) of the action of cyanide on respiration differs from that of carbon monoxide, both accomplish the same results, i.e., cessation of respiration. In both cases it is assumed that the respiratory pigment is inactivated; while, when carbon monoxide is present, carbhemoglobin which is a stable compound of hemoglobin, is formed. In both cases the exchange of O is prevented. When methylene blue is added it is assumed that it takes the place of these poisoned systems, acting as a catalyst by transferring O until normal respiration has again been established.

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and Dr. J. G. Geiger, Director of Public Health of San Francisco for grants to enable me to carry on this work; to Dr. J. M. D. Olmsted, Head of the Department of Physiology, and Dr. C. D. Leake, Head of the Department of Pharmacology of the University of California for valuable technical advice and the loan of apparatus. The assistance of Miss Elizabeth Wood in carrying out these experiments is also acknowledged.

SUMMARY

Injections of methylene blue into rabbits before or after the administration of carbon monoxide gas, showed definitely that methylene blue is an antidote for carbon monoxide poisoning according to previous findings.

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THE CARDIAC OUTPUT IN HYPERVENTILATION BY EXTERNAL ALTERNATING PRESSURE[1]

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It is well known that hyperventilation with pure air results in a depression of the blood pressure in most experimental animals and in a proportion of human subjects. Yandell Henderson (1) described this effect as early as 1905, and from subsequent experiments, largely on animals hyperventilated by intratracheal insufflation, has concluded that it is due to a diminution of the venous return to the heart by a venopressor mechanism. Dale and Evans (2), using similar methods, in 1922 showed by cardiometer experiments that the cardiac output is not significantly altered in acapnia, and indicated that the site of action is in the vasomotor centers of the bulb and spinal cord, causing arteriolar dilatation. Collier, Densham and Wells (3) and others have found that acapnia induced in man by voluntary overbreathing results inconstantly in a fall of blood pressure; Vincent and Thompson (4) concluded that this fall of blood pressure in man, when it occurred, was due to mechanical interference with circulation in the thorax. McDowall (5) in 1930 reported that in a percentage of dogs anesthetized with chloralose no fall or even a rise of blood pressure occurs with hyperventilation, whereas in all dogs anesthetized with ether a fall occurs; he advanced the hypothesis that acapnia has a dual effect on the circulation, i.e., arteriolar dilatation by a central effect, and constriction of smaller and more peripheral vessels by a local effect.

Experimental hyperventilation has been almost exclusively by intratracheal insufflation. Ewing and Janeway (6) in 1914 showed that depressions of the blood pressure similar to those obtained in acapnia produced by intratracheal insufflation could be obtained by the building-up of intrapulmonary pressure without acapnia; these findings were confirmed in the course of the present studies. At least two factors exist which may result in lowered blood pressure with increased intrapulmonary pressure, independently of the degree of acapnia, viz. (a), mechanical impairment of the pulmonary circulation, as suggested by Ewing and Janeway, and (b), reflex cardio-inhibition, as described by Johnson and Luckhardt (7). To

[1] Aided by a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

avoid these effects a respirator of the Drinker (8) type was devised, in which hyperventilation is obtained by alternations of air pressure on the exterior of the animal's body.

By the use of this method of hyperventilation, the cardiac output may be conveniently estimated by application of the Fick principle, and the relation between the blood pressure and the cardiac output studied, and compared with the relation existing in other conditions of lowered blood pressure. Johnson and Blalock (9) have found that hemorrhage and experimental trauma are associated with a decline in the output of the heart which occurs before and to a greater degree than the depression of the blood pressure, while after the injection of histamin the blood pressure declines before and to a greater extent than the cardiac output. These effects obviously depend on the capacity of the arteriolar and capillary bed, as compensatory vasoconstriction occurs in hemorrhage and related conditions, and vasodilatation follows histamin administration. Studies of this relationship between the blood pressure and the cardiac output will be described in seven cases of hyperventilation by external alternating pressure, and in five comparison experiments in which the blood pressure was lowered by *a*, hemorrhage, *b*, ligation of large veins, and *c*, histamin administration, the cardiac minute volume being measured in each case by application of the Fick principle.

METHODS. Dogs were used in all experiments, and were anesthetized by sodium-barbital, given intravenously in doses of approximately 275 mgm. per kgm. body weight. Sufficient heparin, amounting to 30 mgm. per kgm., was given to render the blood incoagulable. Continuous blood pressure tracings were made by connection of a cannula in the left carotid artery directly to a mercury manometer.

a. *Hyperventilation series.* In seven experiments the animal was shaved about the neck and shoulders, washed and dried, and placed in a metal cylinder 30 cm. by 15 cm. in diameter, the open end of which was closed by a thick rubber cuff sealed about the neck by several layers of adhesive tape (see diagram of apparatus, fig. 1). The air-tight chamber thus formed was connected by a side-arm to two Palmer respiration pumps, which were operated synchronously, at approximately 115 revolutions per minute. For the determination of the oxygen consumption a Benedict-type spirometer was used. Because of the inertia of its floating chamber and counterbalance, the spirometer could not freely follow the rapid respiratory movements; hence a breathing chamber with one wall a rubber diaphragm was devised to take up the oscillatory movement, while the position of the spirometer float continued to indicate the oxygen consumption. The chamber used was a metal cylinder 19 cm. by 22.5 cm. in diameter, connected directly to the tracheal cannula, containing soda-lime on its floor and in a gauze bag suspended over the tracheal connection, and closed at the top

by rubber sheeting 1 mm. thick. A mixture of approximately 60 per cent oxygen in air was circulated through the entire apparatus by a small motor blower. Repeated tests showed the apparatus air-tight, and that the rubber diaphragm returned to its normal position after oscillatory movements. Samples of the mixed venous blood were obtained from the right heart by means of straight silver cannula 20 cm. long, passed down the right jugular vein from above the rubber cuff about the animal's neck, samples of arterial blood were taken through a T-cannula in the right carotid artery. The arteriovenous oxygen difference was obtained by estimations in duplicate

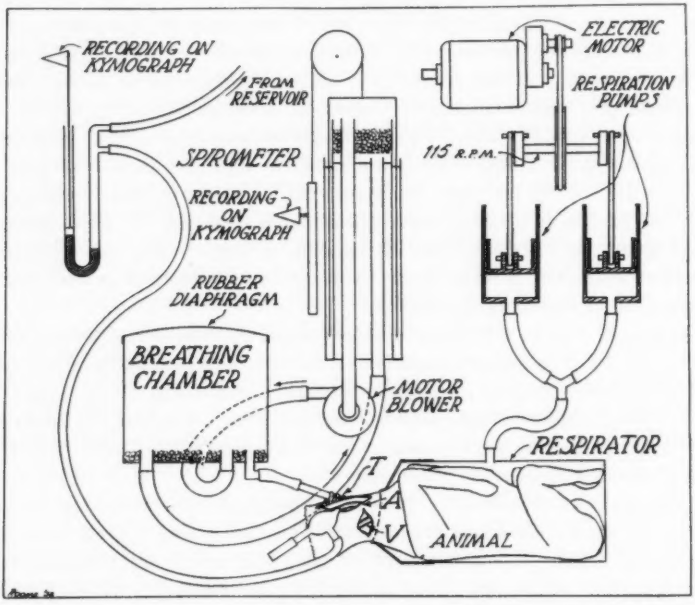

Fig. 1. Diagram of apparatus. *T*, trachea; *A*, carotid artery; *V*, jugular vein

on a Van Slyke manometric blood-gas machine, and the cardiac output calculated by the Fick formula, viz.:

$$\frac{\text{oxygen consumed per minute}}{\text{arteriovenous oxygen difference}} = \text{volume of blood passing through the lungs per minute}$$

$$= \text{cardiac minute volume (less the coronary circulation)}$$

From examination of table 1, which contains the results of this series of experiments, it will be noted that the extent of fall of the blood pressure

varied considerably in the different animals although the blood carbon dioxide levels were similar. In five of the seven cases the blood pressure fell to levels of from 64 to 95 per cent of the "normal,"[2] while the cardiac output fell to a less degree, being from 84 to 110 per cent of the "normal" values. In two cases (527 and 561) a slight but definite increase in the blood pressure occurred, while the cardiac minute volume remained at 96 and 99 per cent of the "normal" values. One of these animals was hyperventilated a second time, and a still greater rise of the blood pressure occurred; the administration of approximately 5 per cent carbon dioxide in air caused a depression of the blood pressure. Periods of apnea of from 65 to 175 seconds following the cessation of hyperventilation were observed in all cases.

TABLE 1

Results of hyperventilation series

EXPERIMENT NO.	WEIGHT OF ANIMAL	"NORMAL" VALUES			PERIOD OF HYPERVENTILATION	VALUES DURING HYPERVENTILATION			BLOOD PRESSURE DURING HYPERVENTILATION, AS PERCENTAGE OF "NORMAL" BLOOD PRESSURE	CARDIAC OUTPUT DURING HYPERVENTILATION, AS PERCENTAGE OF "NORMAL" CARDIAC OUTPUT
		Blood pressure	Cardiac output	CO_2 content		Blood pressure	Cardiac output	CO_2 content		
	kgm.	*mm. Hg*	*cc./ min.*	*vols. per cent*	*minutes*	*mm. Hg*	*cc./ min.*	*vols. per cent*		
515	6.45	157	1257	42.7	20	121	1240	20.4	77	99
518	6.65	99	810	40.4	14	83	730	25.0	83	90
521	4.9	110	590	37.5	14	80	650	15.3	73	110
526	5.55	142	860	33.8	19	135	895	20.5	95	104
527	4.6	109	214	33.4	10	111	206	21.3	102	96
561	6.0	147	1254	39.1	15	148	1243	22.0	101	99
564	4.5	143	505	45.7	21	91	425	17.3	64	84

b. Control experiments to hyperventilation series. In experiment 521 (see table 1), recovery was permitted after 18 minutes of hyperventilation; the blood pressure returned to a level (114 mm. Hg) 104 per cent of the "normal," 24 minutes after the cessation of the artificial respiration, and the cardiac output was at this time 95 per cent of the "normal" value.

In experiment 533, after a marked fall of the blood pressure was found to occur with hyperventilation with the air-oxygen mixture, the ventilation was repeated after the removal of the soda-lime from the spirometer circuit and the addition of a sufficient quantity of carbon dioxide to the gas mixture to maintain the blood pressure at approximately its normal level (see fig. 2). Simultaneous blood pressure readings and blood carbon diox-

[2] Throughout this paper, "normal" refers to the previous resting level of blood pressure or cardiac output of the anesthetized animal.

ide determinations made in this experiment are listed in table 2; it will be noted that while there was a marked fall of the blood pressure during hyperventilation resulting in acapnia, essentially no change in the blood pressure occurred when the blood carbon dioxide content was maintained at its normal level.

Histologic studies of the lungs of three dogs after periods of hyperventilation of from 45 to 210 minutes revealed no evidence of acute emphysema, hemorrhage or edema.

These experiments indicate that in hyperventilation by external alternating pressure (1), there is no permanent circulatory or pulmonary damage, and (2), the blood pressure depression is prevented by the maintenance of the blood carbon dioxide level at its normal, indicating that the change is due to chemical and not to mechanical action.

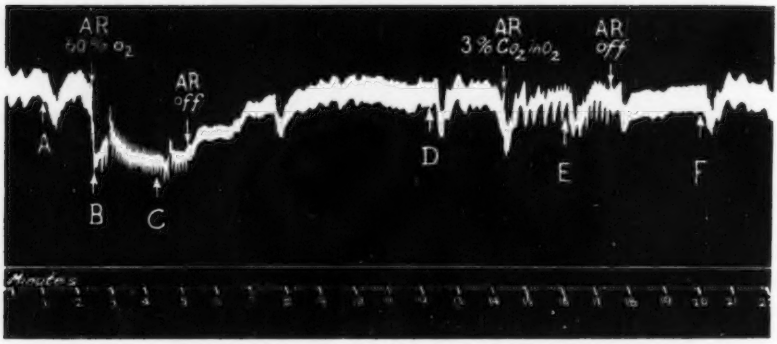

Fig. 2. Kymograph tracing of arterial blood pressure, experiment 533. Note the typical blood pressure fall upon hyperventilation with air-oxygen mixture, and the maintenance of the normal blood pressure level after the addition of carbon dioxide to the gas mixture. Letters *A-F* refer to table 2.

c. *Comparison experiments. Hemorrhage.* In two cases, after estimating the "normal" output of the heart in a similar manner to that described above, the blood pressure was slightly reduced by repeated small bleedings from a carotid artery, and the cardiac output again determined. The results are contained in table 3, and confirm the findings of Johnson and Blalock (9), inasmuch as the cardiac output fell proportionately more than the blood pressure.

Venous ligations. Two dogs were prepared by isolating the right and left femoral and subclavian veins and the abdominal vena cava immediately below the renal veins. The blood pressure and the cardiac output were determined as in the previous experiments, before and shortly after the ligation of these vessels. The results are contained in the second section

of table 3, and show that a similar relation between the cardiac output and the blood pressure exists after venous ligations as after hemorrhage, i.e., that the cardiac minute volume is reduced proportionately more than the blood pressure.

TABLE 2

Control experiment 533

	POINT ON TRACING, FIG. 2	CO_2 CONTENT	BLOOD PRESSURE
(a) Hyperventilation with 60% oxygen in air			
		vols. per cent	*mm.Hg*
"Normal"	A	39.3	116
20 sec. after beginning hyperventilation	B	27.9	69
2 min. after beginning hyperventilation	C	26.5	68
(b) Hyperventilation with approx. 4% CO_2 added to air-oxygen mixture			
"Normal"	D	39.3	111
2 min. after beginning hyperventilation	E	38.4	109
2½ min. after cessation of hyperventilation	F	39.5	108

TABLE 3

Comparison experiments

EXPERIMENT NO.	WEIGHT OF ANIMAL	"NORMAL" VALUES		TIME INTERVAL	VALUES AFTER PROCEDURE		BLOOD PRESSURE AFTER PROCEDURE, AS PERCENTAGE OF "NORMAL" BLOOD PRESSURE	CARDIAC OUTPUT AFTER PROCEDURE, AS PERCENTAGE OF "NORMAL" CARDIAC OUTPUT
		Blood pressure	Cardiac output		Blood pressure	Cardiac output		
(a) Hemorrhage								
	kgm.	*mm.Hg*	*cc./min.*	*minutes*	*mm.Hg*	*cc./min.*		
516	22.7	156	3450	25	140	2480	90	72
520	6.45	84	1390	17	72	935	86	67
(b) Venous ligations								
522	8.95	105	1420	7	105	1105	100	78
525	9.5	115	2330	14	117	1785	102	76
(c) Histamin administration								
523	9.2	163	2640	18	94	1920	58	73

Histamin administration. One animal was given a small dose of histamin in aqueous solution subcutaneously. The blood pressure was continuously recorded and the cardiac minute volume was estimated before the administration of the drug and after the blood pressure had fallen to

57.5 per cent of its "normal" level, at which time the output of the heart was reduced to 73 per cent of the "normal." This is the reverse relationship of blood pressure and cardiac output to that observed in hemorrhage or venous obstruction, as was demonstrated by Johnson and Blalock.

Discussion. The experiments described indicate that in the acapnia induced in dogs anesthetized with sodium-barbital by hyperventilation by external alternating pressure, the blood pressure is often depressed, but the response is irregular and the blood pressure may remain at or slightly exceed the normal level. These findings correspond with those of McDowall (5) on dogs anesthetized with chloralose and hyperventilated intratracheally

The cardiac output was in all cases proportionately less affected than the blood pressure. This relationship of blood pressure and cardiac output is the same as that observed (by Johnson and Blalock and corroborated here) to exist in histamin "shock" and differs from the effect of hemorrhage and of obstruction of the venous return to the heart. It is thought, therefore, that the depression of the blood pressure by acapnia is chiefly by arteriolar dilatation, rather than by mechanical interference with circulation in the thorax or impaired venous return to the heart.

In the animals in which the blood pressure remained at or exceeded the "normal" level during hyperventilation, the output of the heart was not increased; i.e., either the central vasodilator action is inhibited, or there is a compensatory peripheral vasoconstrictor mechanism as suggested by McDowall, rather than compensation by increased output of the heart.

SUMMARY

1. Methods for the induction of experimental acapnia in dogs by external alternating (positive and negative) pressure, and for the estimation of the cardiac output by the Fick formula, during hyperventilation, are described.

2. Most of the animals tested suffered a fall of blood pressure when hyperventilated in this manner; certain animals maintained a normal blood pressure in the presence of acapnia.

3. The cardiac output was less reduced than the blood pressure, in all cases in which the latter fell; this indicates that the depressant action of acapnia is by arteriolar dilatation.

4. No evidence was found of a primary impairment of the venous return to the heart by action on a venopressor mechanism by the acapnia so induced; it is likely, however, that interference with the pulmonary circulation occurs in intratracheal insufflation methods.

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THE EFFECT OF HEATING EGG WHITE ON CERTAIN CHARACTERISTIC PELLAGRA-LIKE MANIFESTATIONS PRODUCED IN RATS BY ITS DIETARY USE[1]

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The failure of early feeding experiments with purified or restricted rations employing egg white or "egg albumin" as the sole protein, and usually relatively poor in vitamin content were reviewed by Bateman (1916, 1917) who reported his own extended study of the utilization of egg proteins by dogs, rabbits, rats and man. He noted particularly a striking diarrhea which usually occurred on feeding well supplemented diets rich in fresh or hydrated dried egg white and was preventable by certain treatments of the egg white.

A variety of rations rich in egg white, raw or cooked, has been found to be rapidly fatal to rats, especially to young rats (Friedberger, 1926a; Friedberger and Seidenberg, 1927; Tscherkes, 1927; Friedberger and Abraham, 1930; Parsons, 1931; and Maignon and Chachine, 1931). If this early nutritive disaster was avoided, there was a period of growth and well-being in rats fed egg white in rations of whole egg or in supplemented rations with varying percentages of fresh or dried egg white, raw or slightly cooked, but this period of a few weeks was followed by the appearance of a characteristic pellagra-like disorder (Boas, 1924a, b; Friedberger, 1926b; Scheunert and Wagner, 1927; Friedberger and Seidenberg, 1927; Boas, 1927; Scheunert, Schieblich, and Wagner, 1928; Stenqvist, 1928; Friedberger, Abraham, and Seidenberg, 1928; Findlay and Stern, 1929; Parsons, 1931; Bliss and Thomason, 1930–31; and Fixsen, 1931).

After some preliminary discordant results, the German laboratories cited were in agreement in showing that raw whole fresh egg began to lose its toxicity on heating at about 70° and was incapable of causing the pellagra-like symptoms if heated at 100° for from 3 to 30 minutes. Boas (1927) and Findlay and Stern (1929), feeding only cooked egg white in their ex-

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periments, considered that the drying process created the toxicity of egg white, but only if the drying took place before cooking. Boas, Findlay and Stern, Bliss and Thomason, and Parsons (loc. cit.) found certain substances such as yeast, egg yolk and beef liver to be potent in preventing or curing the pellagra-like lesions if certain proportions were maintained between the amounts of egg white and the curative substances.

The present experiments were undertaken to determine the comparative effects of heating Chinese dried egg white, moist fresh and storage egg white, and moist egg white dried in the laboratory, in their respective dermatitis-producing capacities.

EXPERIMENTAL. *Animals and their care.* Both albino and hooded rats were used as the experimental animals, the former in larger proportion because of their greater sensitiveness to the effects of dietary egg white. The routine of their care has been described (Parsons, Smith, Moise, and Mendel, 1930).

Rations. The modifications of Sherman's and of Steenbock's stock rations with increased butter fat previously reported (Parsons, 1931) were again employed and are designated as stock rations I and II respectively. A further modification of each of these in which dried skim milk powder and fresh butter replaced equivalent amounts of the dried whole milk of the previous rations, and the total butter fat of the rations was increased by an additional 10 per cent are designated as stock rations III and IV respectively. Five per cent of dried cooked beef liver was also incorporated in each of these latter rations (stock rations V and VI).

Experimental egg white ration B previously reported was continued and others in table 1 were added.

Foodstuffs and their preparation. Fresh eggs less than 24 hours old were provided from experimental flocks by the Department of Poultry Husbandry of the University of Wisconsin. For one experiment (lot 24) the eggs were brown shelled, from so-called American breeds, being nearly exclusively from Plymouth Rock hens. The fresh eggs for the remaining experiments were white shelled from Leghorn hens. As it was necessary to use eggs from more than one pen for each lot, strict diet control was not maintained.

Cold storage eggs were obtained on the market and were used three to four weeks after they were graded and packed. They were in a fair condition, some discoloration of the white and a tendency for the yolk to deteriorate rapidly being noted. The commercial dried egg white was an imported Chinese fermented variety. The process of manufacture as described by Blomberg (1932) includes a short spontaneous "fermentation" of the fresh egg white with the production of acid, a subsequent addition of small portions of alcohol and ammonia, and the rejection of a bottom layer before the drying of the product. Egg white from fresh and cold

storage eggs was dried on shallow trays before a fan in this laboratory at *cir.* 23° without additional treatment. These preparations were fed either raw in their dried form, or cooked at certain temperatures after being reconstituted by a thorough mixing with water in the proportions present in fresh egg, 3 to 4 hours of soaking at room temperature being allowed before cooking, to insure the complete absence of lumps. In two experiments, soaking was continued for 3 and 5 days, respectively, at 3°. The Chinese egg white in solution showed an acidity of approximately pH 6.4. Both the reconstituted Chinese and laboratory-dried egg white and egg white

TABLE 1

Plan of experimental egg white rations

	RATION											
	B	H	I	J	K	L	M	N	O	P	Q	R
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Egg white (dry basis) or derivatives	66	40	40	30	30	20	20	10	10	10 +20	20 +30	20 +40
Salt mixture***	4	4	4	4	4	4	4	4	4	4	4	4
Yeast****	10	10	0	0	0	0	0	0	0	0	0	0
Wheat embryo	10	10	0	0	0	0	0	0	0	0	0	0
Sucrose (heated or unheated)**	10	36	56	66	0	76	0	86	0	66	46	36
Corn starch (heated or unheated)**	0	0	0	0	66	0	76	0	86	0	0	0
Alcoholic extract beef liver** (expressed as equivalent weight of dried liver)	0	0	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)

3 to 5 drops of cod liver oil daily were fed separately.

** Sucrose or corn starch carried the alcoholic extract of beef liver when this appeared in the ration. Extract of yeast was substituted for extract of liver in the ration of 3 rats in lots 51 and 52.

*** Osborne and Mendel, 1919, p. 572.

**** Northwestern Yeast.

from fresh and cold storage eggs were cooked in a double boiler over water, the temperature of which was so regulated that the egg white reached the desired temperature in approximately 4 minutes (never less than 3 or more than 5). The egg white was either cooled quickly at this point or heating was continued for a given time, with vigorous stirring. When the interval of heating exceeded one hour, the temperature was maintained in an electric oven after the initial heating. Commercial cornstarch and a highly refined sucrose were incorporated into the ration either as purchased or after heating for 7 days at 100°. Beef liver prepared as previously de-

scribed (Parsons, 1931) was shaken out with ether and a 60 per cent alcoholic extract was prepared from this and from yeast, respectively, according to the method of Sherman and Sandels (1930–1931) for yeast.

Plan of experiment and criteria of degree of detoxification of egg white. Rats, usually from 30 to 35 grams in weight, were fed a well supplemented ration, presumably adequate, but high enough in raw Chinese egg white (66 per cent, ration B) so that the characteristic pellagra-like condition would be produced speedily in spite of the presence of the protective yeast and wheat germ. Inasmuch as a high percentage of young rats, weaned from ordinary stock diets, die within a few days on this ration (Parsons, 1931), they were protected in this experiment, by the inclusion of a small amount of liver (5 per cent) in the ration of the mother, this addition being discontinued at the time the pregnant females were isolated, and not resumed until after the young were weaned. When the body weight became stationary on ration B or dropped slightly and the dermatitis had reached a definite state (grade C described below), 10 to 66 per cent of egg white which had been treated in one of various ways was substituted for the raw Chinese egg white during a 30-day test period. During this time growth might be rapid and the pellagra-like manifestations might disappear entirely or only slight degrees of roughness or thinness of the hair on the back, or of pigmentation might persist (grade A); the condition might improve somewhat, with a resumption of moderate growth, but with the persistence of thinness and raggedness of the hair, particularly on the lips, a residual redness or swelling of the lips and possibly bare areas of skin (B); the condition might remain about stationary with possibly more or less growth but with decided, although not progressive, soreness and swelling of the lips, with some scaliness and bareness of the skin and redness of the paws (C); or the condition might become distinctly worse, the redness and soreness of the lips becoming intense and spreading to the nose and ventral surfaces of the body, the paws and arms becoming bare, edematous and sore, the skin very scaly, pigmented and bare, and emaciation and nerve symptoms becoming pronounced (D).

In this paper the word "toxicity" is used for want of a better term, to denote the tendency of raw egg white to require a greater amount of a protective factor or factors, the larger the amount of egg white incorporated in the diet.

RESULTS. *Incidence of pellagra-like manifestations in relation to diet of mother, body weight at weaning and to sex and variety of rat.* The transmission of a small amount of a protective factor to the young through the placenta or mother's milk (see plan of experiment) proved successful in giving a fairly uniform protection against the usual high mortality found among ordinary weanling rats during the first few days on a ration high in egg white, yet at the same time did not delay the appearance of the pella-

TABLE 2

Loss of toxicity of egg white on heating as measured by the effect on pellagra-like manifestations in rats

LOT NUMBER	TYPE RATION	EGG WHITE	SOURCE AND TREATMENT OF EGG WHITE	GAIN OR LOSS OF BODY WEIGHT IN 30-DAY TEST PERIOD OR BEFORE DEATH	SEVERITY OF CONDITION AT END OF 30-DAY PERIOD OR AT DEATH*****
		per cent		*grams*	
1†	B	66	Fresh, raw, moist	−21†	D†
2	H	40	Fresh, raw, dried	+13 +11 −25	D D D*
3	J	30	Fresh, raw, dried	−22	D*
4	L	20	Fresh, raw, dried	−4 −14 −20 −22 −30	C D D D D
5	N,O	10	Fresh, raw, dried	+27 +18 −14 −2 −1 +11 −7 −18 −34	A B B B C D D D* D*
6	B	66	Fresh, brought to 67°, fed moist	−8 −12 −14	D* D* D*
7	H	40	Fresh, 67° for 10 min., dried	−4 −5	D D
8	H	40	Fresh, 67° for 20 min., dried	+2 +17 +10 −8	C D D D
9	B	66	Fresh, 67° for 1 hr., dried	−6 −16	D D*
10	H	40	Fresh, 67° for 1 hr., dried	+41 +21 +0	B C D*
11	H	40	Fresh, brought to 75°, fed moist	+29 +18 −6 −13 −14 −25	C D D D D* D*
12	H	40	Fresh, brought to 75°, dried	+8 −4	D D
13	B	66	Fresh, brought to 80°, dried	+8 −14	D D*
14	H	40	Fresh, brought to 80°, dried	+7 −3	D D
15	B	66	Fresh, 80° for 5 min., dried	+105 +60	A A
16	H	40	Fresh, 80° for 5 min., dried	+94 +80	A A
17	L	20	Fresh, 80° for 5 min., dried	+59 +26 +21	A B B
18	L	20	Fresh, 80° for 20 min., dried	+25 +30	A B
19	B	66	Fresh, dried, then 80° for 5 min., dried	+117 +108	A A
20	P	10 +20	Fresh, raw, dried Fresh, 80° for 20 min., dried	+44 +41 +9 +9 +3 −18 −31	A A B C C D D*
21	Q	20 +30	Fresh, raw, dried Fresh, 80° for 20 min., dried	−13 −16 −27 −33	D D* D* D*
22	R	20 +40	Fresh, raw, dried Fresh, 80° for 20 min., dried	−24 −27	D* D*
23	R	20 +40	Fresh, 80° for 20 min., dried Fresh, raw, dried	−22 −29 −39	D D* D*
24	H	40	Brown eggs, fresh, 67° 20 min., dried	+46 +65 +63 +51	A B B B
25	B	66	Fresh +0.2 per cent alcohol, dried then 80° for 5 min., dried	+60 +87	A B

24	H	40	Brown eggs, fresh, 67° 20 min., dried	+46 +65 +63 +51	A B B B
25	B	66	Fresh +0.2 per cent alcohol, dried then 80° for 5 min., dried	+60 +87	A B
26	B	66	Fresh +10 per cent alcohol, 80° for 5 min., dried	+97 +91	A A
33	B	66	Storage, 67° for 20 min., dried	+7 −5	D D
44	B	66	Storage, brought to 85°, dried	+58 +56	A A
45	H	40	Storage, brought to 85°, dried	+125 +77 +62	A A A
46	B	66	Storage, dried, then 80° for 5 min., dried	+85 +76	A A
47	B	66	Storage, dried, then 80° for 10 min., dried	+98 +58 +56 +32	A A A A
48	B	66	Storage, dried, then 80° for 20 min., dried	+78 +57 +35	A A A
49	H	40	Chinese, raw, dry	+46 +8 +8 −7	C D D D
50	I	40	Chinese, raw, dry	−35 −35 −37	D* D* D*
51 (x)	J,K	30	Chinese, raw, dry	−2 −19	D D*
52 (x)	L,M	20	Chinese, raw, dry	0 −4 −10 −14 −16	C C D D* D
53	H	40	Chinese, brought to 67°, dried	−1 −22	D D*
54	H	40	Chinese, 67° for 20 min., dried	−7 −22 −23	D D* D*
55	H	40	Chinese, 75° for 20 min., dried	0 −10 −11	D D* D*
56	H	40	Chinese, 80° for 5 min., dried	−4 −4	D D
57	H	40	Chinese, 80° for 10 min., dried	+64 +30	A B
58	B	66	Chinese, 80° for 20 min., dried	−†† −†† +5 +0 −2 −6 −18 −32	D D D D D D* D* D*
59	H	40	Chinese, 80° for 20 min., dried	+72 +67	A A
60	B	66	Chinese, 80° for 1 hr., dried	0 −14 −17	D D* D*
61	B	66	Chinese, 80° for 1½ hr., dried	+55 +35 +20 −7	A B D D
62	B	66	Chinese, 80° for 3 hr., dried	+133 +130 +66	A A A
63	L	20	Chinese, 80° for 3 hr., dried	+70 +59	A A
64	B	66	Chinese, brought to 85°, dried	−14 −21	D* D*
65	H	40	Chinese, brought to 85°, dried	+61 −3	C D
66	B	66	Chinese, 85° for 20 min., dried	+71 +65 +63 +47	A A A A
67	B	66	Chinese, soaked 3–5 days, 3°, then 80° for 20 min., dried	+107 +81 +62 +37 +129 +50 +19	A A A A B B B

***** D denotes most severe symptoms, A, least severe. For fuller description see plan of experiment. Letters in column 6 are listed for the different rats in the same order as the weights for the same individuals in column 5. An asterisk denotes the death of the animal before the expiration of the 30-day test period.

† For the record of other rats receiving this same ration from weaning, see text.

†† These two rats were placed on the test diet before the appearance of symptoms, for a more crucial test.

x Three rats from these lots were fed an alcoholic extract from yeast instead of from liver.

gra-like condition necessary for testing the toxicity of the egg white. All of the rats used in the following experiments were produced on stock rations V and VI containing 5 per cent of liver. Only 5 per cent of these rats failed to slacken in growth and develop the pellagra-like symptoms in a few weeks. In order to save the expense of the ration, these were killed except for a few which were kept to test the resistance of this small group. These survivors were found to develop the usual symptoms, also, only more slowly than the others.

In a series of 553 rats, those with a body weight of less than 35 grams at the time they were put on ration B, had stopped growing and showed a severity of manifestations graded as "C," at an average of 51 days; those 35 to 40 grams in weight, at an average of 55 days; and those 40 grams or more in weight, at an average of 60 days. Of the whole group, the albino rats, averaging 35 grams body weight when put on ration B, were ready for the test diets at an average of 51 days; the hooded rats averaging 36 grams body weight, at an average of 57 days; the males with an average body weight of 36 grams, at an average of 49 days; and the females with an average body weight of 34 grams, at an average of 57 days. A similar difference between the response of males and females was observed by Boas (1927).

Influence of temperature on toxicity: raw undried fresh egg white vs. heated fresh egg white. No improvement in the condition of a standard test animal (lot 1, table 2) was obtained by substituting fresh egg white (calculated on a dry basis) for dried Chinese in ration B, so it seemed desirable to make the test more crucial by feeding the fresh egg white from weaning, using litter mates in a control experiment with the Chinese egg white ration. Growth was rapid in the animals fed fresh egg white and diarrhea was seldom observed in the first period, but definite sore mouths and other manifestations appeared in these rats in from 50 to 60 days, practically coincident with the same symptoms in the control experiment. Two of these animals on fresh egg white were used for test doses in another experiment, but the remaining 3 died on the 73rd, 92nd and 105th experimental day, respectively, with exaggerated nerve and dermal manifestations (see fig. 1 and chart 1) and, in one case, severe diarrhea.

In testing the effect of heating on the toxicity of egg white, it was found that a temperature of 67° failed to change fresh egg white sufficiently even after an hour's time (lot 9), to permit recovery of animals on it, when used, dried, as 66 per cent of the ration. Fed at 40 per cent (lot 10) it led to some improvement in one rat although another rat died on the 30th day. In order to be sure that the poor results were not simply due to the dried form of the egg white, fresh egg white, which had been brought to 67° in 4 minutes, was fed moist to a group of 3 rats (lot 6). The jelly-like texture was typical of many egg dishes prepared for human consumption. All 3

rats became steadily worse and died within the test period. Even 80° was ineffective (lot 13) if the egg white was merely brought to this temperature, but 5 minutes at 80° changed the egg white so that, in both rations B and H, it led rapidly to recovery (lots 15 and 16).

Fig. 1

Fig. 2

Fig. 1. This shows characteristic acute nerve symptoms in a rat (no. 873) fed raw fresh egg white as 66 per cent (dry basis) of ration B. This kangaroo posture with the weight resting mainly on the tail, the back sharply bent and the arms jerked convulsively backward, was assumed whenever the rat attempted to reach upward. The dermal manifestations were much more severe in this animal before death, although bare, edematous forepaws, swollen sore lips, thin hair, deeply pigmented epidermis and crusted eyelids were present at this time.

Fig. 2. Albino rats E1789 and E1808 died on the same day having been fed their test diets for 25 and 19 days respectively. Rat E1789 (above) received ration R in which 40 per cent was fresh raw dried egg white and 20 per cent fresh egg white cooked for 20 minutes at 80° and dried. Rat E1808 (below) received the same type of ration but 40 per cent consisted of the cooked egg white and only 20 of the raw. Both rats showed equally severe manifestations which included thinness of hair, bareness of head, legs, ventral surfaces and lower back, extreme soreness of mouth, nose, eyes and fore legs, edema of fore paws, scaliness of skin and deep pigment on the back. The tail of E1789 was hard, dry and blackened at the tip. In this experiment no beneficial influence is seen of feeding fresh cooked egg white in the proportion of either one half or twice the amount of raw in the diet.

Influence of type of egg white: different breeds of hens, cold storage eggs, Chinese dried egg white. Egg white from fresh brown Plymouth Rock eggs, heated for 20 minutes at 67° gave measurably greater improvement (lot

24), than similarly treated egg white from fresh white eggs (lot 8), or storage eggs (lot 33).

Since cold storage eggs were easily available throughout the year, the most extensive series of tests were made on these, but only a few of the experiments in that series are reported in table 2, inasmuch as the results

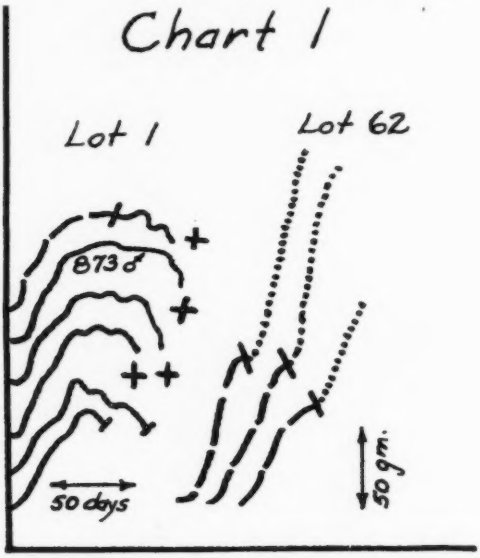

Chart 1. The curves at the left show the results of feeding a ration of moist raw fresh egg white (lot 1). Whether the feeding of the fresh egg white was begun after the pellagra-like condition was established on raw Chinese dried egg white, or from weaning, nutritional failure resulted. (See fig. 1 for illustration of the nervous disorder in rat 873.) Two animals from this group were later changed, before the disorder became too severe, to other test rations with resulting complete cures of the pellagra-like condition. The others died with severe symptoms.

The curves at the right indicate the rapid recovery made by three rats in which the pellagra-like condition was first established on a raw Chinese egg white diet, followed by a change to Chinese egg white which had been thoroughly cooked.

— — — Ration B with raw Chinese dried egg white (66 per cent)—basal ration.

———— Ration B with fresh raw egg white (lot 1).

---------- Ration B with Chinese egg white heated at 80° for 3 hours (lot 62).

\+ Rat died.

were almost exactly identical in regard to changes in both weight and symptoms of the rats with those on fresh eggs treated with a comparable degree of heat. One higher temperature, 85°, was used, and found to be effective if the egg white merely attained it (lots 44 and 45).

Chinese dried egg white, fed at a 66 per cent level (lots 58 and 60), wa

still strongly toxic after 20 minutes or even an hour of heating at 80°, and not all of the animals recovered on egg white heated at this temperature for 1½ hours (lot 61). Inasmuch as animals fed fresh or storage egg white heated for only 5 minutes to the same degree made as good a record as the latter, it is evident that it requires about 18 times as long to detoxify Chinese egg white at 80° as these other two types. As many as 8 animals were tested on the 66 per cent concentration of the Chinese egg white heated at 80° for 20 minutes and all showed definite decline. Although two of these animals were changed to the test diet while still apparently normal, the change did not delay the appearance of the pellagra-like manifestations at the usual time. Heating for 3 hours at 80° or for 20 minutes at 85°, however, rendered the Chinese egg white harmless in the diets in which it was fed (see lots 62, 63, and 66, and chart 1).

Hypotheses for differences between fresh and Chinese dried egg white. That the process of drying, in itself, was not responsible for the striking differences between fresh and Chinese dried egg white was clearly demonstrated by the closely similar responses given by the rats fed cooked fresh egg white, cooked cold storage egg white, and cooked dried fresh or cooked dried cold storage egg white prepared in the laboratory (lots 15, 19 and 46), in contrast with cooked Chinese dried egg white (lots 56, 58 and 60). Thus, fresh egg white dried before a fan was undoubtedly superior to Chinese fermented dried egg white and equal to undried egg white in ease of detoxification by heat.

The addition of alcohol during the manufacture of Chinese dried egg white might conceivably be a factor in the different behavior of the two. However, fresh egg white dried after the addition of 0.2 per cent of its weight of 95 per cent ethyl alcohol, approximately the amount reported (Blomberg, 1932) to be added to Chinese fermented egg white, seemed to be nearly as readily detoxified by heat, after reconstituting, as raw fresh egg white or dried fresh egg white. (Compare lot 25 with lots 15 and 19.) An amount of 95 per cent ethyl alcohol equivalent to 10 per cent of the weight of the fresh egg white was added to another sample just before cooking and was found not to hinder the rate of detoxification of the egg white by heating (lot 26).

That a difference in concentration of a toxic factor in the two types of egg white when raw was not responsible, was indicated by finding that when Chinese egg white and dried fresh egg white were fed raw at various levels the latter was fully as toxic as the former. (Compare lots 49 and 2; and 52 and 4.)

Boas reported (1927) that fresh egg white provides sufficient protective factor against about an equal amount of Chinese dried egg white in the diet. It has already been pointed out that raw fresh egg white produces the same type of pellagra-like lesions in rats that Chinese dried egg white

produces but it seemed possible that on heating fresh egg white, a protective factor might become effective to a markedly greater extent than in Chinese dried egg white. Accordingly, a series of rations was fed in which the ratio of the percentage of raw fresh egg white to cooked fresh egg white (both fed dry) in the diet were: 40 to 20, 20 to 30, 20 to 40, and 10 to 20. Fresh egg white cooked at 80° for 5 minutes and for 20 minutes, respectively, were used in the various rations. All these forms of egg white were also fed separately at various low levels, in control experiments. In doing this it was necessary to change the source of the vitamin B complex from the yeast and wheat embryo used in rations B and H to some source such as the marmite used by Boas in which vitamins B and G are present but the factor preventing egg white injury is apparently negligible. An alcoholic extract of liver was accordingly used in rations I to R inclusive. Some difference in the effect of these supplements at even a 40 per cent level of egg white can be seen in lots 49 and 50. On the whole, little evidence was obtained of a factor present in fresh egg white and protective against injury from dried egg white. Cures were apparently at first obtained from the inclusion of 20 per cent of cooked fresh egg white (fed dried) in ration P with 10 per cent of raw dried egg white, but it soon became evident that 10 per cent of raw egg white alone gave very irregular results, and while extreme manifestations and even death occurred on it, in other cases improvement was seen which was nearly as decisive as when the supplement of cooked egg white was included. Moreover on the latter ration itself, some distinct failures were seen. (Compare lots 20 and 5.) It seemed to be a more crucial experiment than the above, to feed the raw dried egg white at a concentration of 20 per cent on which 5 rats had failed to improve (lot 4), and to incorporate 30 and 40 per cent of cooked fresh egg white (fed dried) in this ration (lots 21 and 22). The response was practically identical from all the rats fed 20 per cent of raw dried egg white whether cooked fresh egg white was included in the ration or not, no improvement being obtained in any of the animals. If there was a protective factor present in cooked fresh egg white dried before feeding, it was too slight to be demonstrated by feeding double the amount of "fresh egg white" found by Boas to be effective (see fig. 2).

During some digestion experiments on egg albumin, Yaichnikov (1930) observed that the protein had not gone completely into solution at the end of 9 hours. This suggested to us that one of the factors in the resistance of dried Chinese egg white to detoxification by heat might be a relative slowness of hydration. In two experiments, egg white was accordingly soaked for a 3 and a 5 day interval, respectively, at 3° and then heated at 80° for 20 minutes. The results from 7 rats gave evidence that both samples were considerably less toxic than Chinese egg white soaked only 2 to 4 hours before being subjected to the same amount of heating. (Compare lots 67 and 58.)

Question of influence of food intake. In general, the animals which recovered from the pellagra-like condition and gained in weight ate more food than the animals which gradually became worse, lost weight and died, as was to be expected. A study of the detailed food intake records however revealed that failure of the animals to recover could not possibly be attributed in general to a failure to eat the ration. In the first place, there was nearly always an initial gain in weight when the rats were first put on the test diets on which they later failed. With cases of extreme soreness of the lips, the food intake often decreased somewhat toward the end of the 30-day period, but, in a number of instances, rats within a few days or even a few hours of death with the exaggerated symptoms of egg white injury ate fully as much food per kilogram of body weight as rats which were improving rapidly. Under these conditions, extensive paired feeding was not essential but a few control experiments were performed. For example, one rat when ready to be put on a test dose was given, for 15 days, 5 grams a day of a ration containing some cooked fresh egg white known to be non-toxic (lot 16). Although the rat could only barely maintain its weight on this amount of ration, nearly the lowest intake observed in the negative experiments, the dermatitis disappeared and new hair reappeared on the lips as rapidly as in the rats eating nearly double this portion. When the rat was allowed an ad libitum intake of food, hair speedily reappeared on the back, also, and the weight increased on an average more than 6 grams a day for 14 days.

Discussion. From these results it is apparent that the toxicity of egg white, manifested by its tendency to produce a pellagra-like condition in rats, in rations not harmful when other proteins are used instead of egg white, is a general property of native egg white, and not merely the result of desiccation. Chinese fermented dried egg white does, however, differ from fresh egg white in being strikingly more resistant than the latter to detoxification by heat, and this fact probably accounts for Boas' results which showed a difference between fresh and Chinese dried egg white, heated for a short time to prevent diarrhea, which led her to conclude that drying creates toxicity in egg white. It is not clear why Chinese dried egg white is resistant to detoxification by heating. It has been shown by these experiments that it is not due to the following: the drying process as such; either a higher concentration of a toxic factor or a lower concentration of a protective factor than is present in fresh egg white; a possible staleness of the egg white at the time of drying; nor the small amount of alcohol that is added after the fermentation process, unless, possibly, long storage after alcohol treatment and drying has an influence. It is possibly due to the type of egg. Whatever the difference involved, it was not duplicated by the process of drying egg white in this laboratory before a fan, although Boas' laboratory-dried egg white behaved more like Chin-

ese dried egg white than like fresh egg white in her experiments. However, as the process of heating was not being specifically studied by her, it is possible that the degree of heat was not precisely uniform in the two sets of rations. From the present experiments it seems probable that the relative slowness of the Chinese dried egg white to go into solution before cooking, partly accounts for its resistance to detoxification.

Our results are in agreement with those of Friedberger which show that coagulated egg white may still carry toxicity. The disagreement between Friedberger on the one hand, and Scheunert, Widmark, Stenqvist and their associates on the other, as to the precise extent of detoxification of whole egg by heating it to 70° and 100° for 3 to 10 minutes may very well rest on differences in the various laboratories in regard to the methods of heating the egg and taking the temperatures, to the variety of eggs, and to the effectiveness of the yolks as a protective factor.

The suggestion of Scheunert and Wagner (1927) that the loss of hair, the soreness of lips and jaws and the swollen eyelids in the rats on raw egg might be attributable merely to a mopping of the head with the fore paws wet with the sticky liquid egg seems improbable inasmuch as the same symptoms were elicited in the present experiments with egg white cooked as long as one hour at 80° and fed dry in rations indistinguishable in consistency from those preventing the lesions.

The pellagra-like condition shown to result from inclusion of egg white in the diet appears to involve an interrelation between a positive toxicity and a *relative* absence of a protective factor, rather than a simple deficiency. If egg white is kept at a constant concentration in the diet, a varying lack of the protective factor can be made to appear as a simple deficiency; but with the protective factor constant, injury can be decreased or increased in a roughly quantitative manner by varying the concentration of raw egg white or the degree of cooking to which it is subjected. It should be stated, however, that this gradation occurs in these experiments, as in those cited by Boas (1927), only within the range of certain proportions between the toxic egg white and a protective factor. It should also be noted that the rats show an individual variation in their ability to cope with moderate degrees of toxicity, so that, near the point of detoxification in a series, distinctly irregular results tend to appear. A concentration of 10 per cent of egg white apparently gives more irregular results than 40 and 66.

These experiments do not fully answer the question as to what degree of heat treatment is necessary to render egg white a satisfactory source of protein in standard purified basal diets, inasmuch as a certain amount of the factor protective against egg white injury was probably included in even the most restricted of the test rations used in these experiments, in the attempt to furnish an abundance of the recognized vitamins. A strict limitation of the sources of the vitamin B complex in purified rations might

conceivably reduce the concentration of the factor or factors protective against egg white injury below that used in these experiments, and a slightly unfavorable effect of even fairly well cooked egg white might confuse the results of the experiment. Moreover, the advisability of using egg white as the source of protein in experimental diets might be questioned if it is replaced in the control diet by a different protein, as in the experiments of Tilden and Miller (1930–31).

SUMMARY

The injury manifested by a pellagra-like condition in rats resulting from the inclusion of egg white in certain well supplemented rations is not dependent upon the drying process but accompanies the use of fresh undried egg white in the ration. It is absent if sufficient heat treatment is given to the egg white from either fresh or storage eggs, egg white dried in the laboratory or Chinese fermented dried egg white. A temperature of 67° which produces a jelly-like texture in egg white typical of many egg dishes commonly used in human dietaries is not, however, sufficient, even when continued for one hour, to completely detoxify any of these four types of egg white mentioned above. Some even higher temperatures held for a short time are insufficient. Egg white from fresh or cold storage eggs, heated either at once or after being dried in the laboratory and moistened again, is almost entirely detoxified by a temperature of 80° maintained for 5 minutes. Egg white from the brown shelled eggs used in one experiment was somewhat more readily detoxified by heat than that from white shelled eggs. Chinese fermented dried egg white requires for detoxification approximately eighteen times the length of heating at 80° that the other three types require, a relative slowness in hydration of the former apparently being partially responsible for the difference.

The injury appears to involve an interrelation between a positive toxicity and a *relative* absence of a protective factor.

Thoroughly heated egg white subsequently dried, although not toxic in itself, contains little or no protective factor potent against raw egg white also contained in the diet.

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ARTIFICIAL INDUCTION OF OVULATION AND OESTRUM IN THE EWE DURING ANOESTRUM

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The sexual season of the ewe varies considerably in length in different breeds of sheep. In some breeds, the non-pregnant ewe has a continuous series of oestrous cycles throughout the year, whereas in certain wild species of sheep, the ewe may have but a single oestrum each year (Marshall, 1910). However, in most of the domestic breeds, the sexual season is five to six months in length. It is characterized by a number of oestrous cycles recurring at about seventeen-day intervals (McKenzie and Phillips, 1930). During the remaining part of the year, from March to September, the ewe enters into a state of anoestrum. Although there are indications that the reproductive organs are not entirely quiescent throughout this period (Cole and Miller, 1931) it is nevertheless true that outward manifestations of heat are lacking.

Data are presented in this paper regarding the amount of the gonad-stimulating hormone of mare serum necessary to induce ovulation and oestrum in ewes during the anoestrous period. Also, data are presented to show that the interval of time between injections is of importance in this species. A preliminary experiment indicated that a single injection, or injections on several consecutive days, would not induce oestrum. In this experiment, seven Romney ewes were given from 500 to 1500 rat units of gonad-stimulating hormone.[1] Three were given a single injection and the remaining four received doses on five consecutive days. Some of the injections were given subcutaneously and others intravenously. Regardless of the method of administration, vaginal smears indicative of oestrum or early post-oestrum were obtained on the second or third day following the first injection. None of the ewes showed evidence of oestrum when tested with males. These ewes were injected during mid-anoestrum but did not

[1] We have arbitrarily defined the rat unit of the gonad-stimulating hormone of mare serum as the amount which will produce, in a group of six rats, an average of from three to ten mature follicles or corpora for each immature female rat tested and autopsied five days after the injection, and half of which amount will fail to consistently produce a vaginal smear of oestrum in another group of six rats (Cole, Guilbert and Goss, 1932).

come into heat before the normal time for the occurrence of oestrum in this breed.

EXPERIMENTAL. The methods used in the experiments to follow may be briefly explained. In every instance, untreated serum from pregnant mares was used as the source of the gonad-stimulating hormone. This serum had been previously filtered through a Berkefeld filter and assayed with immature female rats. It contained 50 rat units of gonad-stimulating hormone per cubic centimeter. We consider this a very potent serum with our method of assay, although serum with twice this concentration, or perhaps even greater, may be occasionally encountered. The material was injected subcutaneously in the axillary region where the skin is practically free of wool. The sheep in the first two experiments were old Shropshire ewes (five to eight years old). Had the experiments been negative, the use of animals of this age could be criticized. However, the high degree of success in producing ovulation in the first experiment and the close agreement of the results in the second and third experiments indicate that the age of the animals did not influence the results to a marked extent. In the third experiment, young Romney ewes from our breeding flock were used.

Effect of gonad-stimulating hormone upon the ovary during anoestrum. The preliminary experiment had given indirect evidence that there had been an effect upon the ovary because of the marked vaginal reactions produced. Therefore, 21 ewes were injected and autopsied four days later to determine the ovarian response. This time of autopsy was selected as all of the ewes in the preliminary experiment had shown metoestrous smears on the fourth day following the injections. Four other ewes served as non-injected controls. Part of the ewes were given a single injection and part were injected on three consecutive days. The amount injected was varied in order to determine the response at several dosage levels. Details in regard to the size of the dose administered and the results obtained are given in table 1. The ewes were injected on June 11th, about three months before the normal time for the first oestrum to occur in the breed used.

The data on ovulation is based upon the occurrence of rupture points rather than the finding of ova in the oviduct. Inasmuch as the rupture point is very conspicuous in the ewe, this would appear to be a relatively safe criterion of ovulation. We realize that a premature luteinization of the follicle might interfere with the escape of the ovum. However, in the next experiment we have a better criterion of ovulation, namely, the inception of pregnancy following breeding. Thus, we felt that sectioning of the oviducts to locate the ova was not justified.

We were surprised to find that not only was there ovarian development but that 16 out of 19 ewes receiving 50 rat units or more had ovulated. None of these ewes had come into oestrum previous to autopsy. We commented upon this experiment elsewhere during its progress (Cole, Guilbert

and Goss, 1932) and stated that we believed it improbable that ovulation was produced unaccompanied by heat. We thought at the time that perhaps the ram might become impotent during the anoestrous period of the ewe but later experiments have not sustained this belief.

TABLE 1

Induction of ovulation in ewes during anoestrum by subcutaneous injection of the gonad-stimulating hormone

The ovaries were weighed after storage in Bouin's fixative.

DESIGNATION OF EWE	SIZE OF DOSE	NUMBER OF DOSES*	TOTAL AMOUNT ADMINISTERED	WEIGHT OF OVARIES	NUMBER OF RUPTURED FOLLICLES	REMARKS
	R.U.		*R.U.*	*gms.*		
29	Control			1.4	None	Many small follicles
36	Control			2.6	None	Many small follicles
43	Control			2.3	None	Many small follicles
48	Control			3.9	None	Many small follicles
41	7	3	21	1.8	None	Many small follicles
42	15	3	45	1.5	None	Many small follicles
31	50	1	50	2.9	1	
32	100	1	100	2.5	3	
44	35	3	105	1.9	None	Many small follicles. Also one slightly enlarged follicle
33	150	1	150	1.9	2	
45	50	3	150	1.9	None	Many small follicles
46	65	3	195	2.5	1	
34	200	1	200	2.3	2	
47	80	3	240	3.4	1	
35	250	1	250	2.6	1	
37	300	1	300	2.6	2	
49	100	3	300	2.7	2	
38	400	1	400	3.2	2	
50	135	3	405	2.9	2	
51	165	3	495	2.7	3	
39	500	1	500	1.9	2	
52	333	3	999	5.8	3	
40	1000	1	1000	27.3	None	Many large follicles
53	660	3	1980	10.4	1	
54	1500	3	4500	3.1	3	

* Where three doses were given they were administered on three consecutive days.

Table 1 shows that 50 R. U. was the smallest dose to produce ovulation. Only two ewes were included at lower levels (ewes 41 and 42). However, it is likely that this is approximately the minimal dose for there was not a marked increase in the size of the ovary until 20 times this amount had been injected. The number of ruptured follicles does not appear to be corre-

lated with the size of dose. As a matter of fact, where ovulation has occurred at all the number of ruptured follicles is comparable to the number found in ewes following normal oestrum. We have found a similar circumstance when gonad-stimulating hormone of mare serum was injected into immature female rats. To our knowledge super-ovulation cannot be

TABLE 2

Induction of oestrum in ewes during anoestrum with the gonad-stimulating hormone

DESIGNATION OF EWE	SIZE OF INITIAL DOSE	SIZE OF 2ND DOSE ADMINISTERED 16 DAYS AFTER INITIAL DOSE	NUMBER OF DAYS BETWEEN 2ND DOSE AND OESTRUM	RESULT OF BREEDING DURING INDUCED OESTRUM
	R.U.	*R.U.*		
55	150	250	No oestrum*	
56	150	250	1	Pregnant
57	150	250	No oestrum	
58	150	250	4	Pregnant
59	150	250	No oestrum	
60	250	250	3	Pregnant
61	250	250	2	Pregnant
62	250	250	2	Pregnant
63	250	250	7	Pregnant
64	250	250	2	Pregnant
65	350	250	No oestrum	
66	350	250	14	Pregnant
67	350	250	5	Pregnant
69	350	250	No oestrum	
70	450	250	4	Pregnant
71	450	Control	No oestrum	
72	450	Control	No oestrum	
73	450	Control	No oestrum	
74	450	Control	Bred 18 days after initial injection	Pregnant
75	50	Control	No oestrum	
76**	250	Control	No oestrum	
77**	250	Control	No oestrum	
78**	250	Control	No oestrum	
79**	250	Control	No oestrum	
80**	250	Control	No oestrum	

* "No oestrum" refers to the absence of oestrum for 31 days after initial injection.

** These ewes were given 50 rat units of Theelin with the initial dose of gonad-stimulating hormone.

produced with this substance and this is the only clear-cut difference between the administration of gonad-stimulating hormone and the implanting of anterior pituitary glands. Smith and Engle (1927) showed clearly that super-ovulation could be produced by the latter method in the rat. Table 1 further indicates that the size of the dose rather than the number of doses determines as to whether ovulation will occur.

Induction of oestrum in ewes during anoestrum. The experiments already cited give evidence that a single injection or several injections on consecutive days of gonad-stimulating hormone would not bring ewes into oestrum. Further, the preliminary experiment indicated that a single injection would not hasten the time of the first oestrum of the sexual season. Two lines of attack were next undertaken to induce oestrum during the anoestrous period. First, a second dose of the hormone was administered after an interval corresponding to approximately the length of the oestrous cycle (17 days) had elapsed following the first injection. Secondly, the effect of injecting Theelin simultaneously with gonad-stimulating hormone was studied. The dates on which injections were made were June 20th and July 6th. The normal time for the first oestrum of the sexual season to occur for the breed used is September. Table 2 shows that a second injection 16 days after an initial injection brought on oestrum in from 1 to 14 days in ten out of fifteen ewes. The amount given the first time was varied, but five times the minimal dose was given in each case in the second injection (the minimal dose was approximated from data given in table 1). All of the ewes in which oestrum was induced were bred and became pregnant. There is a considerable variation in the time elapsing between the second injection and the expression of oestrum. Ewe 66, for example, did not come into oestrum until 14 days after the second injection. This is perhaps most easily explained by assuming that the injection started the rhythmical events associated with the cycle and that the oestrum occurring at this delayed time was spontaneous and only indirectly the result of the injection. The fact that ewe 74, which received only one injection, came into oestrum 18 days after the injection might be explained in like manner. It is apparent, however, that a single injection will seldom bring on oestrum for this occurred in only one out of ten ewes in this experiment.

As regards the second phase of this experiment—the administration of Theelin and gonad-stimulating hormone simultaneously—the results were entirely negative. Further experiments are necessary in order to determine if larger amounts of the female sex hormone are effective.

Effect of gonad-stimulating hormone upon the time of the first oestrum of the sexual season. This experiment is very similar to the previous one. There are, however, some modifications. The time of the first oestrum of the sexual season in 5 untreated controls was accurately determined. Furthermore, the second injection was made 14 instead of 16 days after the first. Only two ewes came into oestrum as a result of the second injection. This may have been due to the shorter interval of time between the two injections. Thirteen of the ewes received a third injection 16 days after the second. Eight of these came into oestrum on the second day following injection (table 3). One other came into oestrum 17 days after the third injection. Thus, there were two ewes in this group (ewes 1389 and 5790)

in which the response was delayed until a time corresponding to the length of one cycle had elapsed following the injection. The explanation given for the delayed oestrum in two cases of the preceding experiment may also apply to these. The four remaining ewes came into oestrum at the normal time for the breed.

Definite data cannot be given at this time regarding the number of animals becoming pregnant during the induced oestrum following breeding.

TABLE 3

The effect of the gonad-stimulating hormone upon the time of the first oestrum of the sexual season in mature Romney ewes

DESIGNATION OF EWE	DATES ON WHICH LAST INJECTIONS WERE MADE*	DATE OF FIRST OESTRUM
1150	Control	September 10
1386	Control	September 14
4798	Control	September 17
1400	Control	September 28
1481	Control	October 15
1256	Control	Later than November 1
1241	July 26	July 31
1389	July 26	August 11
1257	August 11	August 13
1391	August 11	August 13
1470	August 11	August 13
1471	August 11	August 13
1474	August 11	August 13
969	August 11	August 13
1148	August 11	August 13
1157	August 11	August 13
5790	August 11	August 28
770	August 11	September 19
967	August 11	September 30
973	August 11	October 4
1480	August 11	Later than November 1

* All of the injected ewes received the first and second doses on July 12 and July 26 respectively. All but two (1241 and 1389) received a third injection on August 11. The dose consisted of 250 R.U. of gonad-stimulating hormone.

Ewes 1241, 1471, and 969 came into oestrum at a later date. Thus, the fertility was not as high as in the preceding group.

SUMMARY AND CONCLUSIONS

Sixty-one ewes were injected with the gonad-stimulating hormone of mare serum in an attempt to induce ovulation and oestrum during anoestrum. Ovulation was produced by a single injection of 50 R. U. or more of gonad-stimulating hormone. However, using this procedure, the ewes did not come into oestrum. Further, if injections were made on several

consecutive days the ewes did not exhibit oestrum, but if a second injection followed the first after an interval of 16 days (the oestrous cycle in sheep is about 17 days long) both ovulation and oestrum were produced. A high percentage of fertile matings resulted. In another group in which the second injection was made 14 instead of 16 days after the first, only 2 out of 15 ewes came into oestrum. A third injection 16 days after the second resulted in 8 out of 13 ewes coming into oestrum on the second day following injection. The evidence leads us to the conclusion that the cyclic phenomena associated with the oestrous cycle are activated by a single injection of gonad-stimulating hormone, but that a second stimulus (injection) must be applied after a specific time interval in order that all the events associated with oestrum may be manifested. Therefore, the results show that the administration of sufficient amounts of the gonad-stimulating hormone at proper time intervals will result in oestrum, ovulation, and impregnation following breeding during the anoestrous period.

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STUDIES OF HOMEOSTASIS IN NORMAL, SYMPATHECTOMIZED AND ERGOTAMINIZED ANIMALS

I. The Effect of High and Low Temperatures

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It has long been recognized that if complex organisms are to exist and lead an independent life, certain constant conditions must be maintained in their internal environment. Cannon (1929) suggested that the specific term "homeostasis" should be employed to designate these steady states which are so essential to the well-being of an animal. The maintenance of homeostasis is secured by the operation of certain complex physiological agencies which are brought into play when any alteration of external or internal conditions threatens to upset the balance existing in the internal environment. So efficient are these agencies in the normal animal that it can withstand quite drastic variations in its surroundings without suffering any permanent ill effects. It was pointed out by Cannon (1929) that the sympathetic division of the autonomic nervous system plays an important rôle in helping to maintain homeostasis when certain circumstances arise which tend to alter the internal environment of an organism. On the other hand, if no such emergencies arise and the animal leads a sheltered life, the entire sympathetic nervous system can be removed without interfering in any way with its well-being (Cannon, Newton, Bright, Menkin and Moore, 1929).

The object of the present investigation has been to obtain more information concerning the part played by the sympathetic nervous system in enabling an animal to adjust itself to changes in both its external and internal environment. The method employed was to make an experimental and statistical study of three groups of animals, normal, sympathectomized and ergotaminized, and compare their ability to withstand variations in temperature and oxygen concentration in their external surroundings, and to lowering of blood sugar and blood pressure in the internal environment. The technique used in removing the sympathetic nervous system was that described by Cannon *et al.* (1929), except that the superior and inferior cervical ganglia and the sympathetic fibers in the neck were left intact.

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THE EFFECT OF HIGH AND LOW TEMPERATURES. Warm-blooded animals maintain a relative constancy of their body temperature, when exposed to a hot environment, by dilatation of their skin vessels and outpouring of sweat; or, when exposed to a cold, by vasoconstriction, an erection of dermal covering such as feathers and hairs, and discharge of adrenin with attendant increase of blood sugar and more rapid oxidation (Cannon, Querido, Britton and Bright, 1927; McIver and Bright, 1924). There are other activities such as panting or shivering, but those mentioned above involve the sympathetic system and indicate its possible importance in thermal homeostasis.

In the study of the reaction of animals to temperature extremes in the external environment, chief emphasis has been placed on a comparison of their ability to withstand heat, since Cannon, Newton, Bright, Menkin and Moore (1929) had previously observed that sympathectomized cats are more susceptible to cold than normal cats. Because the present investigation was partly a statistical study, however, it was thought desirable to obtain information regarding the effects of low as well as high temperatures on the reaction of a number of sympathectomized animals, and also of normal and ergotaminized animals. Changes in body temperature together with any superficially observable reactions have been used to judge the animals' ability to withstand changes in the temperature of their surroundings.

METHODS. Cats were used for all experiments. The animals were trained to lie quietly on the table or they were held in the lap during temperature determinations, since it was found that emotional excitement or restlessness caused a rise in their temperature. The clinical thermometer was in all cases placed in the groin and ten minutes was allowed for registration. Usually several readings were taken at room temperature before exposure of the animal to heat or cold. In the exposure to heat the animals were placed in individual small cages in a room which varied from 39.45 to 40.30°C. on different days. The animals were left in this environment from 1 to 2.5 hours, depending on their reaction. During this period the body temperature was determined several times and the responses of the animals, such as panting or increased respiration and salivation, were observed. A similar procedure was carried out in the cold room where the temperature varied between 8.00 and 9.00°C. In some cases observations were made in the hot and cold rooms on cats while normal; a few days later ergotamine was administered to these animals, and their reaction to heat or cold again observed; at a third stage complete sympathectomy was performed and they were again tested, thus permitting a comparison of the three states on the same animal. In all experiments in the hot or cold room normal cats were exposed simultaneously with the sympathectomized and in most cases with the ergotaminized animals.

EXPERIMENTAL RESULTS. *Control experiments at room temperature.* Inguinal temperatures were taken on twenty-four normal cats at room temperatures varying between 22.00 and 25.00°C. and the average normal value was found to be 38.65°C. with a range between 38.00 and 39.10°C. Slight fluctuations were observed when temperatures were taken throughout the day, but these as a rule did not amount to more than 1.00°C. The lowest daily temperature was usually found in the morning and the highest in the afternoon.

Twenty-four temperature readings were made also on twelve sympathectomized cats at room temperature on different days. The average was practically the same as that for normal cats, namely, 38.60°C. with a minimum of 38.10° and a maximum of 39.15°C. But the behavior of these animals differed from that of the normal animals in that they invariably sought the warmest place in the room, e.g., near the radiator or in a box on the table under an incandescent lamp. It was noticed in several of these animals that after lying for about an hour exposed to the heat of the lamp, they became warmer by 0.60 to 0.85°C. and that a fall to the previous level occurred about an hour after the light had been extinguished. Frequently on cool days shivering was observed in sympathectomized animals at room temperature when the normal animals showed no signs of discomfort.

Ergotamine (Stoll, 1921) was used to block the motor or positive action of sympathetic nerve impulses (Dale, 1906), i.e., the vasoconstrictor and the pilomotor. The behavior of ergotaminized animals was studied under the test conditions already mentioned and was compared with that of the normal and sympathectomized.

Numerous observations have been made on the effect of ergotamine on body temperature and heat production, but there are some discrepancies in the observed facts. Marine, Deutch and Cipra (1927) found a fall in body temperature and heat production of normal rabbits after the administration of ergotamine. Similar observations on body temperature have been made by Abderhalden and Wertheimer (1927), Otto (1928), Rigler and Silberstern (1927), Bouckaert and Heymans (1929), and Brink and Rigler (1929) in rats, rabbits and cats. On the other hand, Bouckaert (1926) observed no temperature reduction in a normal dog with ergotamine, although it produced a rapid fall of heat production in an animal which had previously received thyroxin. Confirmatory studies on normal dogs have been reported by Youmans and Trimble (1930) who gave 0.25 mgm. ergotamine intravenously and failed to obtain any decrease of heat production. A rise of the body temperature of rabbits and cats was found by Githens (1918) after ergotoxine, although in rats and mice it caused a marked lowering. The discrepancies in these results are explained by Youmans and Trimble as being chiefly due to the size of the dose and mode of administration of the drug, larger doses and subcutaneous administra-

tion being more effective in producing a fall of temperature than small doses (0.25–0.50 mgm.) and intravenous injection.

In this investigation ergotamine tartrate "Sandoz" (ampules for pharmacological use only) was employed. The dose most commonly used was 0.5 mgm. per kgm. injected intramuscularly. Quite often just after the injection the animals appeared nauseated and sometimes vomited. At the same time, soon after the injection, signs of sympathetic activity, such as increased heart rate, erection of hair on the back,and dilated pupils were also noted in a few cases. A similar stimulating action of ergotamine on the sympathetic of unanesthetized dogs was observed by Farrar and Duff (1928), and Dale (1906) reported stimulation of vasoconstrictors as the first effect of ergotoxine on anesthetized cats. In the present work the phase of nausea and hyperactivity of the sympathetic disappeared within 30 to 60 minutes after the injection. The only other visible effect of the injection was an occasional slight muscular weakness of the hind legs.

The effect of ergotamine on the temperature of normal cats was studied by taking readings throughout the day, before and at intervals after the injection of the drug, while the animals were kept at room temperature. In some a slight rise (0.30 to 0.60°C.) took place immediately after the injection and persisted for about one hour. It was followed by a fall of about 0.60 to 0.85°C., which lasted five or six hours. In other animals the slight hyperthermia after ergotamine was not observed; the only effect was a lowering of the temperature.

Experiments in the hot room. It was found that the normal cats were able to withstand exposure to high temperatures much better than either the ergotaminized or sympathectomized. In many cases the normal animals showed no outward manifestation of discomfort save a somewhat faster respiration. In others there was a marked acceleration of respiratory rate (from 20 to 120 respirations per minute), and sometimes panting occurred. In a number of these normal cats sweating on the foot pads was observed, those of the hind feet usually becoming moist before the fore feet. The panting usually ceased as soon as the animal was removed from the hot room, and the respiration quickly returned to normal. A rise of body temperature was observed in all the normal animals while in the hot room. During the first hour the rise was greatest, usually from 1.00 to 1.35°C. The average increase in fourteen experiments was 1.40°C., with a maximum of 1.90° and a minimum of 1.00° (see table 1). After removal from the hot room the body temperature gradually returned to normal in 1 to 2 hours.

The behavior of the sympathectomized animals in the hot room was more striking than that of the controls. These cats were less able to withstand the heat. The increase of respiration was more marked than in the normals, panting was always observed, and it usually occurred continu-

TABLE 1

Response of normal, sympathectomized and ergotaminized cats to exposure to a high temperature

CAT	DATE 1932	BODY TEMPERATURE BEFORE ENTERING HOT ROOM	TIME IN HOT ROOM		TEMPERATURE OF HOT ROOM	BODY TEMPERATURE JUST AFTER LEAVING HOT ROOM	MAXIMAL RISE IN TEMPERATURE WHILE IN HOT ROOM	BEHAVIOR IN HOT ROOM
					Control cats			
		°C.	hours	minutes	°C.	°C.	°C.	
89	March 15	38.45	2	00	39.45	40.10	1.65	Panting, sweating
28	March 15	38.40	1	50	39.45	38.90	1.00	No panting, slight sweating
88	March 15	39.00	2	15	39.45	40.00	1.00	Panting, sweating
96	March 24	38.90	1	30	40.30	40.10	1.20	Slight panting, no sweating
97	March 29	38.90	1	00	40.00	40.65	1.80	Slight panting, slight sweating
92	March 30	38.55	1	00	40.20	40.10	1.55	No panting, no sweating
89	April 6	38.50	1	50	40.30	40.40	1.90	Panting, sweating
92	April 6	38.90	1	10	40.30	40.20	1.35	Slight panting, no sweating
30	April 12	38.75	2	00	40.00	40.20	1.45	Panting, no sweating
48	April 20	38.90	2	00	40.20	40.20	1.35	No panting, no sweating
49	April 26	38.70	2	00	40.00	40.05	1.35	Panting, no sweating
45	April 26	39.00	2	40	40.00	40.30	1.30	Panting, no sweating
43	June 22	38.65	2	00	40.30	40.00	1.35	Slight panting, slight sweating
	Average rise						1.40	
					Sympathectomized cats			
95	April 12	38.45	1	50	40.00	40.65	2.20	Marked panting, no sweating
99	April 12	38.50	1	50	40.00	40.65	2.15	Marked panting, no sweating
94	April 12	39.20	2	00	40.00	41.00	1.80	Panting, no sweating
96	April 20	38.60	2	15	40.20	40.80	2.15	Extreme panting, no sweating
99	April 20	38.65	2	05	40.20	40.75	2.05	Extreme panting, no sweating
95	April 20	39.00	2	00	40.20	41.10	2.10	Extreme panting, no sweating
94	April 26	39.10	2	15	40.00	41.00	1.90	Marked panting, no sweating
95	April 26	38.50	2	15	40.00	40.75	2.05	Marked panting, no sweating

TABLE 1—*Concluded*

CAT	DATE 1932	BODY TEMPERATURE BEFORE ENTERING HOT ROOM	TIME IN HOT ROOM		TEMPERATURE OF HOT ROOM	BODY TEMPERATURE JUST AFTER LEAVING HOT ROOM	MAXIMAL RISE IN TEMPERATURE WHILE IN HOT ROOM	BEHAVIOR IN HOT ROOM
Sympathectomized cats—*Concluded*								
		°C.	hours	minutes	°C.	°C.	°C.	
96	April 26	38.65	2	10	40.00	40.90	2.20	Marked panting, no sweating
89	June 22	38.65	2	00	40.30	41.35	2.65	Extreme panting, profuse salivation
96	June 22	38.55	2	00	40.30	42.20	3.65	Extreme panting, profuse salivation
88	June 22	39.15	2	00	40.30	41.35	2.15	Panting, salivation
	Average rise						2.25	
Ergotaminized cats								
29	March 15	38.95	1	50	39.45	41.10	2.20	Marked panting and sweating
89	March 24	39.00	1	45	40.30	42.35	3.35	Extreme panting, sweating, dilatation of pupils
88	March 24	38.65	1	50	40.30	41.45	2.80	Extreme panting, sweating, dilatation of pupils
28	March 24	39.00	1	23	40.30	42.80	3.80	Extreme panting, sweating, dilatation of pupils, collapse
96	March 29	38.40	1	00	40.00	41.15	2.80	Marked panting, sweating
27	March 30	38.55	1	10	40.20	41.45	2.90	Panting, sweating
92	April 12	38.90	1	15	40.00	41.10	2.20	Marked panting, pupils dilated, nearly collapsed
	Average rise						2.87	

ously. Profuse salivation was also noticed in many of these animals and sometimes diarrhea. Sweating (on the foot pads) never took place in any of the sympathectomized cats.

The rise of temperature in the sympathectomized cats (while in the hot room) was more marked than in the normal animals, usually being greater than 1.40°C. in the first hour and considerably more than this at the end of the exposure. The greatest rise observed in a sympathectomized cat was 3.65°C. and the least 1.80°C., the average rise in twelve experiments being 2.25°C. as compared with 1.40°C. for the normal animals (see table 1). It should be noted that the rise of temperature was greatest in the

sympathectomized animals six or eight weeks after the final operation, when they had recovered their normal weight and a regrowth of hair had taken place on the sides and abdomen. For example, see the experiments of June 22, table 1. The normal animal, after two hours of exposure, had a rise of only 1.35°C. and showed only slight signs of discomfort. Three sympathectomized cats in excellent condition exposed for the same length of time showed temperature increases of 2.65, 3.65 and 2.15°C. respectively. These animals panted continuously at a very rapid rate, had profuse salivation and were nearly in a state of collapse when removed from the hot room. On two previous exposures one of these animals showed rises of temperature of only 2.15 and 2.20°C., at which times the hair had only partially grown out and the former weight had not been recovered. The sympathectomized cats recovered their normal temperature usually within 1 or 2 hours after removal from the warm room.

The ergotaminized animals were found to be least able to resist exposure to high temperatures. This observation is not in accord with that of Rigler and Silberstern (1927) who observed a fall of body temperature in animals kept at 29.00°C. after they had received a dose of ergotamine. In the present investigation, although ergotamine at room temperature caused a hypothermia, exposure in the hot room after injection of the drug produced a hyperthermia greater than that seen in either the normal or the sympathectomized animals. During the exposure, which varied from 1 hour to 1 hour and 50 minutes, the temperature rose markedly; the greatest difference was 3.80°C. and the smallest 2.20°C., with an average value of 2.87°C. This increase is 0.62° greater than the average rise in sympathectomized cats and 1.47° greater than that observed in normal animals.

The reactions observed in the ergotaminized animals while in the hot room were more pronounced than those of either of the other two groups. Their respiratory rate rapidly increased to about 250 per minute. Panting was always observed as well as sweating on the foot pads. In a few animals protrusion of the eyeballs and cyanosis of the mucous membranes were also noted. On removal from the hot room the recovery of the ergotaminized animals was considerably slower than that of either the sympathectomized or the normal controls, the hyperthermia in some cases lasting for three to four hours. A typical experiment showing the rise of temperature occurring in two normal, one sympathectomized and one ergotaminized animal during simultaneous exposure in the hot room is seen in figure 1.

In addition to a study of the reactions of three separate groups of normal, sympathectomized and ergotaminized animals, in four cases the different experiments were carried out on the same animal. For example, observations were made on the normal reaction, the reaction after ergotamine and

again after complete removal of the sympathetic nervous system (see fig. 2). In all these animals sympathectomy lessened the ability to control the body temperature when the temperature of the external environment was raised. The administration of ergotamine had an effect similar to sympathectomy, only more marked.

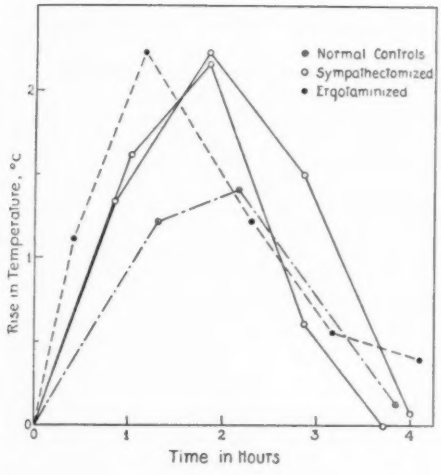

Fig. 1

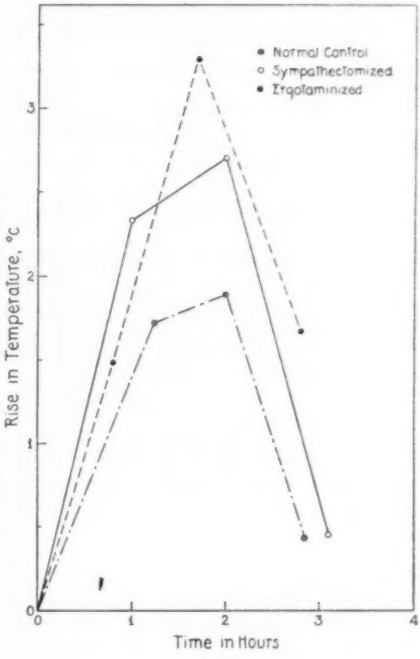

Fig. 2

Fig. 1. Typical experiment in the hot room showing different temperature increases in one sympathectomized, one ergotaminized, and two normal animals.

Fig. 2. Typical experiment on a single animal showing normal reaction to heat and reaction after ergotamine and sympathectomy.

Experiments in the cold room. The temperature of the cold room available for use in these experiments varied between 8.00 and 9.00°C. Normal animals were able to withstand this temperature for 3 to 4 hours without showing any signs of discomfort save spasmodic shivering and occasional erection of the hair on the back. During the exposure there were only slight variations of body temperature, never greater than 0.60°C. in either direction. The usual trend was toward a rise so that most of the normal animals had a slightly higher temperature on leaving the cold room than when they entered it (see table 2 and fig. 3).

TABLE 2

Response of normal, sympathectomized and ergotaminized cats to exposure to a low temperature

CAT	DATE 1932	BODY TEMPERATURE BEFORE ENTERING COLD ROOM	TIME IN COLD ROOM		TEMPERATURE OF COLD ROOM	BODY TEMPERATURE JUST AFTER LEAVING HOT ROOM	MAXIMAL CHANGE IN TEMPERATURE WHILE IN COLD ROOM	BEHAVIOR IN COLD ROOM
Control cats								
		°C.	*hours*	*minutes*	°C.	°C.	°C.	
88	April 15	38.20	2	00	8.00	38.40	0.15 (rise)	Slight shivering, hair rising
30	April 15	38.55	2	00	8.00	38.75	0.20 (rise)	Slight shivering, hair rising
27*	April 19	39.10	2	50	9.00	38.45	0.65 (fall)	Hair standing, shivering
49	April 25	38.10	4	45	8.00	38.50	0.40 (rise)	No shivering
45	April 25	39.10	4	50	8.00	39.20	0.11 (rise)	Slight shivering
30	April 25	38.00	4	00	8.00	38.65	0.65 (rise)	Slight shivering
Sympathectomized cats								
99	April 15	38.35	2	00	8.00	37.50	0.85 (fall)	Marked shivering
95	April 19	38.90	2	05	9.00	37.50	3.60 (fall)	Marked shivering
94	April 19	38.65	1	55	9.00	37.40	1.30 (fall)	Shivering
99	April 25	38.55	4	00	8.00	37.80	0.80 (fall)	Shivering and temperature starts to go up; has fever next day
95	April 25	38.10	4	00	8.00	35.90	2.35 (fall)	Shivering
96	April 25	38.35	4	05	8.00	37.45	0.80 (fall)	Shivering throughout
91	May 17	38.55	4	00	9.00	37.20	1.35 (fall)	Shivering throughout
89	May 17	38.40	3	00	9.00	35.95	2.40 (fall)	Marked shivering
97	May 17	39.05	3	45	9.00	38.35	0.70 (fall)	Shivering (angry when temperature taken)
95	May 17	38.40	4	00	9.00	37.55	1.40 (fall)	Shivering
90	May 17	38.40	2	30	9.00	36.00	2.40 (fall)	Shivering
	Average fall						1.63	
Ergotaminized cats								
27	April 15	38.75	2	35	8.00	37.80	1.00 (fall)	Marked shivering, hair standing
30	April 19	39.10	2	10	9.00	37.35	1.80 (fall)	Slight shivering
48	April 25	38.85	3	30	9.00	37.55	1.30 (fall)	Shivering
	Average fall						1.40	

* Cat came down with snuffles next day.

Sympathectomized cats, on the other hand, were found to be extremely sensitive to cold. As previously mentioned, shivering was frequently observed in these animals even at room temperature, and they always sought out the warmest places. While in the cold room there was continual marked shivering, which began shortly after the start of the exposure.

In all cases a lowering of body temperature was observed in these animals (see table 2 and fig. 3), the average maximal fall in nine experiments

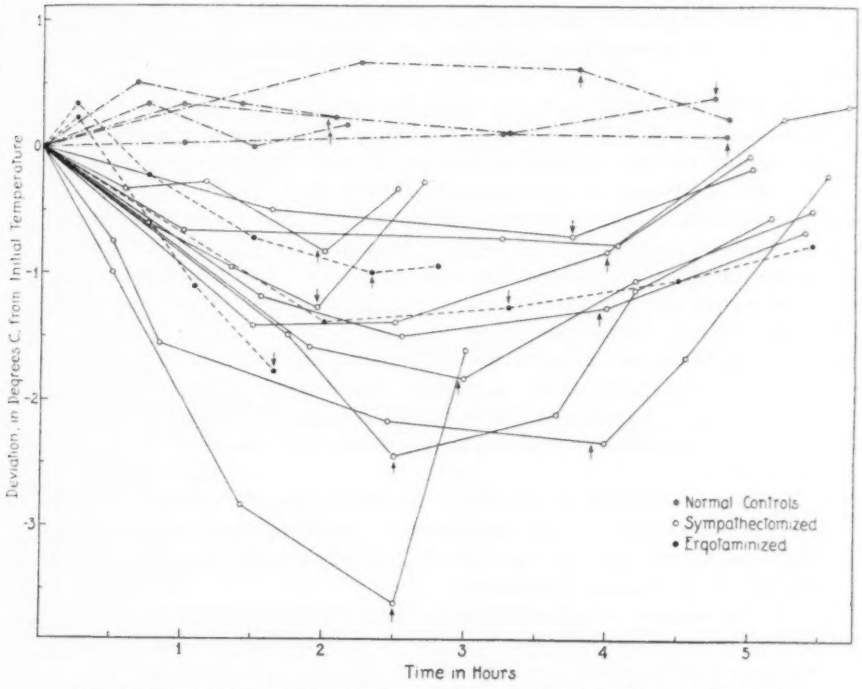

Fig. 3. Chart of all experiments in the cold room showing changes of body temperature in normal, sympathectomized and ergotaminized animals. The arrows indicate time of removal from the cold room.

being 1.63°C. Usually the fall continued for two hours or more, whereupon a constant low level was reached, or sometimes a slight rise occurred before the animal was removed from the cold environment. Signs of discomfort, such as mewing and restlessness, were much more marked in the sympathectomized animals than in the normal.

Ergotaminized cats were also found to be less able to withstand cold than the normal though their reaction was not so striking as that observed

in the hot room. In two of the three animals studied a slight rise of temperature, about 0.30°C., was noted immediately after they were placed in the cold room. This was followed in all three animals by a progressive fall during the first two hours (see table 2 and fig. 3). The average maximal fall was 1.40°C. Shivering was observed in all three ergotaminized cats.

SUMMARY

1. The changes of body temperature were studied in normal, sympathectomized and ergotaminized cats when exposed to warm and cold surroundings.

2. At room temperature the body temperature of sympathectomized and normal animals was practically the same. Intramuscular injections of ergotamine tartrate produced a lowering of body temperature from 0.60° to 1.10°C., preceded in some cases by a slight rise.

3. A distinctly greater rise of body temperature was observed in sympathectomized animals exposed in the hot room (40.00°C.) than in normal animals under the same conditions (see table 1). The obvious reactions, such as panting, increased respiration and salivation, were also more marked in the sympathectomized animals.

4. Ergotaminized animals resembled sympathectomized in their response to heat, except that in all cases the response was more marked, i.e., there was a greater rise of temperature, a greater behavioristic reaction and slower recovery on removal from the hot room (see table 1 and figs. 1 and 2).

5. Exposure of normal animals in the cold room was followed in most cases by a slight rise of body temperature. Sympathectomized animals in this environment showed a distinct fall of body temperature (see table 2 and fig. 3) and marked shivering.

6. The response of ergotaminized animals in the cold room was similar to that of sympathectomized, except that the lowering of body temperature was in most cases not so marked (see table 2 and fig. 3).

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STUDIES OF HOMEOSTASIS IN NORMAL AND SYMPATHECTOMIZED ANIMALS

II. The Effect of Anoxemia

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Numerous investigations on the effect of low oxygen tensions and high altitudes indicate that the mammalian organism can withstand fairly wide variations of the oxygen supply without showing any alarming symptoms. Compensatory reactions, however, may appear with even slight lowering of the oxygen tension. Thus Ellis (1919) observed that at 18 per cent oxygen there was a noticeable increase in the respiratory volume. Further progressive decrease in the concentration (from 18 to 7 per cent) was associated with much greater increases in respiratory volume, and in many cases with hyperpnea (Schneider and Truesdell, 1921; Boycott and Haldane, 1908). Acceleration of the pulse, rise in systolic and pulse pressure, and a moderate terminal drop in the diastolic arterial pressure also take place (Schneider and Truesdell, 1921; Lutz and Schneider, 1919). Experiments on 2,000 humans showed that the average limit of endurance to oxygen deficiency was reached when the oxygen tension was lowered to 7.42 per cent (Schneider and Truesdell, 1921). Oxygen lack in a normal animal is doubtless partially compensated for by increased sympathetic activity, and Cannon (1929) has suggested that the lack may act as a stimulus to the centers controlling the sympathetic system in a manner similar to its action on the respiratory center, i.e., by increasing the acidity of the center. Evidence of increased activity of the sympathetic system in cats during the development of an acute anoxemia is given by the work of Izquierdo (1928). He noted contraction of the spleen and subsequent polycythemia, salivation and erection of the hair, with no emotional disturbance, on subjecting the animals to low oxygen tension.

In the present investigation the point in question was to learn to what degree the sympathetic system is important in maintaining a homeostasis of the oxygen supply when the latter is reduced to the lowest level compatible with life, or, in other words, to learn if normal and sympathectomized ani-

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mals differ in their ability to withstand low oxygen tensions. Several experiments were performed on ergotaminized animals as well.

METHOD. The exposure to low oxygen tension was carried out by placing the animal in a chamber at atmospheric pressure in which the ventilation was gradually changed from the normal to reduced oxygen percentage in an oxygen-nitrogen mixture. The chamber was a large glass bottle of about 20-liters' capacity, the bottom of which had been removed and the edges ground smoothly to fit into a ground circle on a glass plate. The jar was held horizontally in a wooden holder equipped with clamps at one end so that the glass plate could be fastened firmly in place. A rubber stopper with two openings was inserted in the neck of the bottle. A glass tube led through one of these openings from the bottom of the chamber to a suction pump with a constant-pressure valve inserted in the line. The other opening in the stopper led in from a series of three large spirometers which were previously filled with the mixture of oxygen and nitrogen that was to be used.

By starting the suction pump the gas mixture was drawn through the cylinder at a constant rate. As the mixture entered the neck of the bottle, it met a circular plate of metal that caused the inflowing gas stream to spread to all sides. The air was aspirated from the other end of the bottle; thus there was a constant stream running throughout the chamber. The rate of flow was adjusted in all experiments so that a known volume of gas passed from the spirometer in a given time; this was checked by readings of the spirometer scale every few minutes. In some preliminary experiments an animal was placed in the chamber which was ventilated with room air at different rates. The air was analyzed at the end of fifteen to thirty minutes; thus a rate of ventilation was determined which was adequate for keeping carbon dioxide washed out of the chamber during the experiment.

The mixture of gases used was made up in a gas-mixing apparatus and stored under pressure in a cylinder. The gas was then allowed to flow from the cylinder into the series of spirometers, thorough mixing being insured by passing the gas from one spirometer to another. Before the experiment the tubes leading from the spirometers to the chamber were washed out with the mixture and a sample of gas was taken for analysis. The mixtures used contained 6 to 8 per cent oxygen in nitrogen, and usually some carbon dioxide (0.02 to 0.2 per cent) was found.

For an experiment the animal was placed in the jar, and the glass plate, greased with vaseline, was clamped in place over the end of the bottle. The jar was at first well ventilated with room air and a few minutes were allowed for the cat to become quiet before ventilation with the low oxygen mixture was started. Throughout the experiment the changes in respiration and the general behavior of the animal were carefully observed. Just

before the animal was removed from the chamber a sample of the outgoing air was taken for analysis, and was thus designated as final air (see table 1).

The response of normal animals. Insofar as possible the normal animals used for these experiments were chosen from a group which were well acquainted with laboratory routine. They were first accustomed to the chamber by placing them in it on several occasions while it was ventilated with room air. Reduction of oxygen tension called forth two distinct types of reactions. The animals of one group were not disturbed when ventilation with the low-oxygen mixture was begun; they sat quietly throughout the experiment without struggle of excitement. Those of the other group, although remaining quiet during the preliminary ventilation with room air, became greatly excited shortly after the ventilation with low oxygen started. These animals were much less resistant to anoxemia, and since their behavior was different from that of the sympathectomized animals they were not used as controls.

The behavior of the non-excited animals in the chamber was as follows. The first noticeable response was an increase in depth of respiration; this was followed within 3 to 5 minutes by a noticeable increase in respiratory rate. During the next 15 to 20 minutes there was a progressive acceleration of breathing until it reached a maximum rate of 200 to 250 respirations per minute. This rapid rate was in some cases maintained throughout the experiment, while in others it decreased somewhat. Panting and salivation were observed in all the normal animals, the saliva usually starting to flow at the time of maximal respiration. Cyanosis of the tongue and mucous membranes was quite marked and sometimes dilatation of the pupil and protrusion of the nictitating membranes were noted. The normal animals were able to exist for relatively long periods in the low-oxygen atmosphere without the symptoms becoming more marked, provided they sat quietly and did not struggle. Of the five animals that behaved thus one was removed from the chamber 50 minutes and the other four 60 minutes after the start of the experiment. These animals all continued alert, could walk around and showed quick recovery of normal respiration on exposure to room air (see table 1).

Very different was the experience of animals that became excited and struggled during the experiment. Some collapsed after 15 minutes' exposure to low oxygen and none withstood the conditions more than 38 minutes. Just before collapse the respiration became shallow and slow and finally stopped. Quick removal to room air and artificial respiration brought about their recovery.

The response of sympathectomized animals. These experiments were repeated on the sympathectomized animals after they had been used in temperature experiments and were well trained and thoroughly familiar

TABLE 1

CAT	DATE 1932	INGOING AIR		FINAL AIR		DURATION OF EXPERIMENT	MAXIMAL INCREASE IN RESPIRATION PER MINUTE	REACTION OF CAT
		O_2	CO_2	O_2	CO_2			
Normal cats (no struggling while in chamber)								
		per cent	*per cent*	*per cent*	*per cent*	*minutes*		
45	May 26	8.06	trace	6.97	0.90	50 (no fainting)	232	Removed after 50 minutes; no fainting
43	June 20	6.59	0.18	4.57	2.15	60 (no fainting)	140	Removed after 60 minutes; no fainting
44	June 29	6.10	trace	4.89	1.37	60 (no fainting)	200	Removed after 60 minutes; no fainting
40	June 28	6.10	trace	5.12	2.18	60 (no fainting)	220	Removed after 60 minutes; no fainting
42	June 29	6.10	trace	—	—	60 (no fainting)	200	Removed after 60 minutes; no fainting
Sympathectomized cats								
90	May 26	8.06	trace	6.78	1.70	37 (fainted)	240	No struggling after first few minutes, lay quietly till fainted
97	May 26	8.07	trace	—	—	38 (fainted)	228	No struggling, fainted in 38 minutes
96	June 3	6.15	0.25	5.34	0.75	14 (fainted)	120	No struggling, fainted in 14 minutes
88	June 3	6.15	0.25	5.09	0.79	34 (fainted)	160	Moved around jar just before fainting, no struggling
95	June 6	5.95	0.20	4.97	2.20	25 (fainted)	128	Moved around jar just before fainting, no struggling
89	June 20	6.59	0.18	5.28	1.70	30 (fainted)	244	No struggling, fainted in 30 minutes
91	June 21	6.41	0.14	4.73	2.00	38 (fainted)	180	No struggling, fainted in 38 minutes
94	June 21	6.41	0.14	4.84	2.60	22 (fainted)	164	No struggling, fainted in 22 minutes
96	June 28	6.10	trace	—	—	19 (fainted)	162	No struggling, fainted in 19 minutes
90	June 29	6.10	trace	—	—	14 (fainted)	216	No struggling, fainted in 14 minutes
575	June 29	6.10	trace	5.50	1.37	21 (fainted)	210	No struggling, fainted in 21 minutes
96	June 29	6.10	trace	4.70	1.58	28 (fainted)	190	No struggling, fainted in 28 minutes
94	June 29	6.10	trace	—	—	20 (fainted)	120	No struggling, fainted in 20 minutes

with the observers. It should be emphasized that in nearly all cases the sympathectomized cats behaved very quietly during the exposure to low oxygen and did not seem to be greatly disturbed by it. Sometimes the animals moved once or twice around the chamber but usually they remained lying on the bottom. That struggle did not interfere with these experiments is of importance because Freeman and Rosenblueth (1931) demonstrated that in sympathectomized animals a fall of blood pressure and fainting may occur after strenuous effort. In our sympathectomized animals also a great instability on exertion was noticed for some time after the operation. One animal could not walk around without fainting, an-

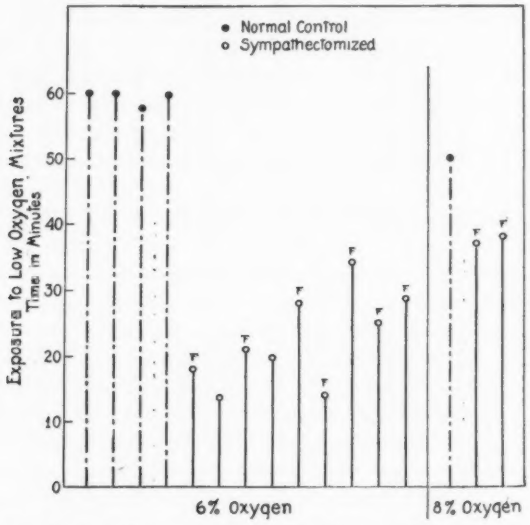

Fig. 1. The resistance of groups of normal and sympathectomized cats to oxygen tensions of 6 to 8 per cent. *F* signifies fainting or collapse.

other in good condition on attempting to jump into its cage collapsed. This sensitivity to exercise disappeared after a week or two and thereafter the animals seemed normal in their ability to withstand a moderate amount of exertion.

The capacity of sympathectomized animals to compensate for a decrease in the oxygen supply was markedly different from that of normal animals (see fig. 1 and table 1). The latter, as noted above, were able to survive exposure to low oxygen tensions for an hour without collapsing. Sympathectomized cats were much more sensitive. In these animals compensatory respiratory reactions were seen, as in the normal; shortly after the ventilation was started the rate was as great as 250 respirations per minute.

After the maximal respiration was reached the animals were unable to adjust themselves to a much longer exposure in the chamber. In no case was there noted a decrease in the respiratory rate and an improvement in the appearance of the animal and the color of the mucous membranes, as had been noted in some of the normal animals. Fainting occurred in all of the sympathectomized cats shortly after the respiratory rate had reached a maximum. The first sign of collapse was seen in a sudden slowing of the respiratory rate, usually the animal thereupon began to mew, made an attempt to move around the chamber and then fell limp. The longest period that any sympathectomized animal withstood the low oxygen tension was 38 minutes, while some of them fainted after 14 minutes' exposure. The sympathectomized animals became alert again shortly after their removal from the low oxygen atmosphere, but it was some minutes before the respiratory rate returned to its normal rhythm.

Only three experiments were carried out on ergotaminized animals and the results are inconclusive. One animal, after receiving 1.5 mgm. of ergotamine, withstood the low oxygen tension for one hour without losing consciousness and at the end of this time, on removal from the chamber, was in good condition. Another, after 2 mgm. of the drug, collapsed at the end of 25 minutes. A third cat remained for only 20 minutes in the low tension chamber before collapsing, but in this case the experiment was complicated by the animal's struggles.

SUMMARY

1. The ability of normal and sympathectomized cats to resist low oxygen tensions was examined.

2. Normal cats which remained quiet throughout the experiment withstood oxygen tensions of 6 to 8 per cent without collapse for at least an hour (see fig. 1 and table 1).

3. If a normal animal became excited and struggled during the experiment, its ability to withstand oxygen deficiency was remarkably lessened. In such cases fainting occurred in a short time.

4. Sympathectomized animals, as a rule undisturbed by the reduced oxygen tension, were unable to resist anoxemia as were the normal non-excited cats. Fainting occurred in all these animals within 15 to 37 minutes after exposure to low oxygen mixtures.

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STUDIES OF HOMEOSTASIS IN NORMAL, SYMPATHECTOMIZED AND ERGOTAMINIZED ANIMALS

III. The Effect of Insulin

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In the course of a study of the regulation of homeostasis in normal, sympathectomized and ergotaminized animals a comparison has been made of their responses to a reduction of the blood sugar.

Cannon and his co-workers (1924) obtained evidence, which was confirmed by other investigators, that lowering of the blood sugar to a critical level caused a hypersecretion from the adrenal medulla. After removal or inactivation of these glands animals became much more sensitive to hypoglycemia. Dworkin (1931) showed that complete removal of the sympathetic nervous system also caused a hypersensitivity to reduction of the blood sugar. Further evidence for the protective action of the sympathico-adrenal mechanism against hypoglycemia was furnished by Burn (1923) who found that ergotamine greatly increased the sensitivity to insulin. In the present investigation a quantitative comparison has been made of the blood-sugar changes and their effects in normal, sympathectomized and ergotaminized animals after the administration of a standard small dose of insulin.

Method. Blood sugar was assayed by means of the micro-method of Folin and Svedburg (1931) for unlaked blood. The figures obtained are lower by about 10 to 20 mgm. than those resulting from methods applied to laked blood, the difference being largely due to absence from the filtrate of reducing disintegration products of the cells. This method is recommended for use especially where low sugar values are to be obtained. The animals were not fed for twelve hours previous to the experiments. Blood samples were taken before the injection of insulin and at half-hour intervals thereafter for a period of 3 to 5 hours. The samples of 0.1 cc. were obtained by nicking the ear vein with a very sharp razor blade while the animals were held on the lap of one of the observers, since in this way emotional disturbance was in most cases entirely avoided. Various doses of insulin were tried from 0.1 to 0.5 unit per kgm., and the latter was finally

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selected as the standard, because it was the smallest dose which as a rule gave critically different results in normal and sympathectomized animals.

Since a study of hypoglycemia with use of the new method of Folin and Svedburg had not yet been reported, special attention was paid to the level at which hypoglycemic symptoms and convulsions occurred. In both sympathectomized and normal animals slight symptoms appeared at 25 to 28 mgm. per cent. At 20 to 25 mgm. per cent marked symptoms (e.g., profuse salivation, disturbance of gait, panting and diarrhea) occurred. Convulsions were observed in a normal animal at 16 mgm. per cent, in three sympathectomized cats at 19, 19 and 12 mgm. per cent, and in two ergotaminized animals at 17 and 15 mgm. per cent.

Blood sugar of normal and sympathectomized animals. Cannon, Newton, Bright, Menkin and Moore (1929) reported blood-sugar determinations in two sympathectomized animals which were practically the same as those found in normal animals. Later Dworkin (1931) published a table of glycemic values on normal animals which averaged about 26 mgm. higher than those obtained on the sympathectomized. In the present investigation, excepting values of 88 mgm. per cent obtained on two normal cats which were used for the first time and were somewhat excited, the normal range was found to be from 62 to 79 mgm. per cent, with an average value of 68 mgm. per cent (see table 1-A). The blood sugar of sympathectomized cats varied between 63 and 72 mgm. per cent, with an average figure of 66. Both the sympathectomized animals and the normal controls used for these experiments had been in the laboratory for some time, were thoroughly familiar with the investigators, and were not excited by the experiment.

The reaction to insulin. The reactions of normal and of sympathectomized animals to small doses of insulin (0.1 to 0.2 unit per kgm.), which are not sufficient to produce hypoglycemic symptoms, are similar. For example, in two normal animals with 0.1 unit per kgm. the blood sugar fell from 86 and 60 to 28 and 36 mgm. per cent respectively, and in two sympathectomized cats from 62 and 73 to 26 and 31 mgm. per cent. The temporal course of the fall after the administration of insulin was nearly the same in all four animals. Administration of larger doses of insulin showed that only when the fall in blood sugar was sufficiently great to cause hypoglycemic symptoms did the normal animals react differently from the sympathectomized.

Eleven experiments were performed on normal animals with the standard dose of 0.5 unit per kgm. Five of these animals had slight hypoglycemic reactions but only one had convulsions (see table 1-A). All the normal animals recovered spontaneously. The average maximal fall in blood sugar in these animals was from 68 to 26 mgm. per cent. It was found that the reaction of the same animal to a standard dose of insulin varied at

TABLE 1

Comparison of the reaction of normal, sympathectomized and ergotaminized cats to a standard dose of insulin (0.5 unit per kgm.)

Blood sugar estimated by the method of Folin and Svedburg

CAT	WEIGHT	BLOOD SUGAR		TIME TAKEN TO REACH LOWEST LEVEL	SYMPTOMS	RECOVERY	REMARKS
		Before insulin	Lowest level after insulin				
A. Normal animals							
	kgm.	*mgm. per cent*	*mgm. per cent*	*hrs. min*			
49	3.3	79	20	2 10	Mewing, salivation, disturbance of gait, no convulsions	Spontaneous	
50	4.2	70	26	2 30	None	Spontaneous	
48	3.1	64	30	2 40	None	Spontaneous	
45	3.7	64	25	2 22	Slight disturbance of gait	Spontaneous	
43	4.9	88*	29	3 25	None	Spontaneous	
35	3.3	88*	27	2 45	None	Spontaneous	
50	4.7	71	36	1 10	None	Spontaneous	
49	3.9	76	35	1 05	None	Spontaneous	
48	2.9	62	17	2 00	Restlessness, salivation, disturbance of gait	Spontaneous	
43	4.5	66	19	2 15	Slight disturbance of gait	Spontaneous	
30	2.7	64	16	2 15	Marked symptoms, collapse, one convulsion	Spontaneous	Blood sample taken just before convulsion
B. Sympathectomized							
91	3.0	72	19	1 45	Marked symptoms, several convulsions	Glucose given	Blood sample taken just after convulsion
89	3.5	65	21	1 35	Marked symptoms, collapse	Glucose given	
96	3.6	68	24	1 45	Marked symptoms, collapse, vomiting, diarrhea	Glucose given	
90	3.5	65	19	1 15	Marked symptoms, convulsions	Glucose given	Blood sample taken just before convulsion
95	3.5	63	12	1 10	Marked symptoms, convulsions	Glucose given	Blood sample taken just after convulsion
94	4.4	63	17	1 30	Marked symptoms, no convulsions	Spontaneous	
C. Ergotaminized							
		Before ergotamine	After ergotamine				Dose ergotamine
							mgm.
49	4.4	75	154	24 3 15	Restlessness, disturbance of gait	Spontaneous	1.75
43	4.5	66	73	15 2 40	Marked symptoms, convulsions	Glucose given	2.0
48	2.9	64	80	17 2 00	Marked symptoms, convulsions	Glucose given	1.5
50	4.7	83	118	Lost Symptoms after 2 00	Disturbance of gait	Spontaneous	2.0

* Cats used for first time, excited.

different times; if the cats were losing weight the sensitivity to insulin became considerably accentuated. This can be explained by a depletion of the glycogen stores since Macleod (1928) found that rabbits when starved went into hypoglycemia with insulin much more readily than when well fed.

Sympathectomized animals were found to be much more sensitive than the normal to the standard dose of insulin (see table 1-B). Although at first the rate of fall of sugar was about the same in the two groups, it reached the lowest level in the sympathectomized considerably sooner than in the normal animals. Of the six sympathectomized cats studied, all showed marked hypoglycemic symptoms and convulsions. The convulsions came on very rapidly after the first appearance of the symptoms of insufficient sugar and in five cases it was necessary to revive the animals by one or several intraperitoneal injections of glucose. The sixth sympathectomized cat, after showing very marked hypoglycemic symptoms, recovered spontaneously. The average fall of blood sugar in the sympathectomized cats was from 63 to 19 mgm. per cent, as compared with 68 to 26 mgm. per cent in normal animals.

The reaction of ergotaminized animals. There is considerable difference of opinion among the numerous investigators who have studied the effects of ergotamine on the blood-sugar level. According to Lesser and Zipf (1923), Hetényi and Pogány (1926), Silberstein and Kessler (1927), Moretti (1927), Seidl (1927), Eda (1928) and Skipner (1929), ergotamine causes a lowering of the blood-sugar level. On the other hand, others have found that the drug either has no influence (Rothlin, 1925; Sakurai, 1926; Pollak, 1929; Youmans and Trimble, 1930) or produces a hyperglycemia (Farber, 1926; Rothschild and Jacobson, 1927; Farrar and Duff, 1928). The discrepancies may partly be accounted for by differences in the size of the dose, mode of administration and species of animal used.

In the present investigation the effect of previous administration of ergotamine tartrate on the hypoglycemia produced by a standard dose of insulin was studied in four animals. In each case the injection of ergotamine (Sandoz), 1.5 to 2.0 mgm. for the whole animal, was followed by a decided increase of blood sugar which lasted for at least 2 hours after the injection (see table 1-C). It should be pointed out that the intramuscular injection of the drug excited the animals and that immediately after the injection mild signs of sympathico-adrenal activity were noted. These, however, usually passed off in the first half-hour, whereas the blood sugar remained high for a much longer period.

In agreement with Burns' experiments on rabbits (1923) it was found that previous administration of ergotamine rendered cats more sensitive to the standard dose of insulin (see table 1-C). Two of the animals went into convulsions and had to be recovered with insulin, while the two others showed hypoglycemic symptoms but recovered spontaneously. The fall

of blood sugar in these animals was greater than in the normal cats and of the same order of magnitude as that in the sympathectomized animals. The time of onset of the hypoglycemic symptoms in the ergotaminized animals was, however, considerably later than in the sympathectomized animals.

SUMMARY

1. No noteworthy difference was found in the average blood-sugar level in normal and sympathectomized animals (see table 1).

2. The fall of blood sugar in normal and sympathectomized animals, after small doses of insulin which do not cause hypoglycemic symptoms, is the same.

3. A standard larger dose of insulin (0.5 unit per kgm.) causes in sympathectomized and normal animals a rapid fall of blood sugar to nearly the level at which hypoglycemic symptoms appear. At this level the blood sugar usually started to rise in the normal animals and there was spontaneous recovery, whereas a further rapid drop to the convulsive level took place in the sympathectomized animals with no spontaneous recovery (see table 1-A and B).

4. Ergotaminized animals were more sensitive than the normal to the standard dose of insulin but not so sensitive as the sympathectomized animals (see table 1-C).

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STUDIES OF HOMEOSTASIS IN NORMAL, SYMPATHECTOMIZED AND ERGOTAMINIZED ANIMALS

IV. The Effect of Hemorrhage

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The preservation of a relatively constant flow of blood to essential organs in animals under standard conditions is one of the most important homeostatic functions. This condition is assured by a fairly steady arterial pressure. Lowering of this pressure by hemorrhage brings into action a protective mechanism which up to a certain point is able to compensate for the fluid lost. Thus Cannon (1923) found that after removal of 20 to 30 per cent of the total blood volume the blood pressure promptly recovers nearly its normal level. That this immediate effect is due to vasoconstriction rather than inflow of fluid from the tissues is shown by the unchanged composition of the blood. It is well known that the sympathico-adrenal mechanism is an important agency in compensating for loss of circulating fluid from the body. Pilcher and Sollman (1914) observed a constriction of the blood vessels after hemorrhage. A similar condition exists if shock is produced by tissue injury (Cannon and Cattell, 1922). Increased output of adrenin and attendant stimulation of the entire sympathetic nervous system also occur when the blood pressure is lowered (Tournade and Chabrol, 1925). Further protective devices against severe hemorrhage which are entirely or partly due to sympathico-adrenal activity are manifest in the contraction of the spleen and its discharge of extra red blood corpuscles into the circulation (Barcroft and Stephens, 1927), and in the faster coagulation of the blood (Gray and Lunt, 1914; Cannon and Mendenhall, 1914).

Since many of the compensatory reactions which enable a normal animal to withstand loss of circulating fluid are controlled by the sympathetic system, it was considered of interest to compare quantitatively the ability of normal and completely sympathectomized animals to meet the test of hemorrhage. At the same time, animals in which the sympathetic system was paralyzed by ergotamine have been studied.

Method. Cats were used for these experiments. The animals were in all cases anesthetized with dial ("Ciba") in doses of 0.5 to 0.7 cc., injected

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intraperitoneally. A catheter was inserted into the trachea for artificial respiration and a cannula introduced into each of the carotid arteries,—one for bleeding, the other for recording arterial blood pressure with a mercury manometer. Five per cent sodium citrate was used as an anticoagulant. The cannula for bleeding was moistened with a heparin solution and the blood was drawn off into a graduated glass cylinder to determine its volume. If the blood was to be reinjected after the hemorrhage, a heparin solution containing 1 mgm. for 3 cc. of blood was first placed in the cylinder. The animals were kept at a nearly uniform temperature on a warming pad throughout the experiment.

Response of normal animals to hemorrhage. A study was first made of the percentage volume of blood which could be removed from a normal animal before the compensatory mechanism, which restores the pressure, failed to respond. The critical level was taken to be the point at which the compensatory response of the vasoconstrictors was very slight or entirely absent and the blood pressure remained at a level of 70 mm. Hg or less. In order to study the responses the animal was bled repeatedly and time was allowed for recovery after each bleeding. Tentative experiments with numerous small hemorrhages led to the adoption of a standard procedure. For the first bleeding a volume of 13 to 15 per cent of the blood was withdrawn, calculated as being 5 per cent of the total weight of the cat. The time taken for this bleeding was kept constant by such pressure on the artery as to release the desired quantity in two minutes. For the second hemorrhage usually 13 to 15 per cent of the blood was again withdrawn, and for the third bleeding 5 to 10 per cent more. The time taken for each of the three bleedings was the same. After each hemorrhage a period of 15 to 45 minutes was allowed to elapse until the blood pressure resumed a constant level. A typical response of a normal animal to the above treatment is seen in figure 1-a. At 3:29, 13.5 per cent of the blood was removed, and in less than fifteen minutes the pressure had returned to its previous level and there remained. At 3:48, 15 per cent of the blood was removed and again in fifteen minutes the blood pressure was restored. A further hemorrhage of 5.4 per cent reduced the pressure to a low level from which no recovery took place. In five such experiments on normal animals the average volume of blood which could be removed before the vasoconstrictors failed to raise the pressure was 38 per cent (see table 1). In four of these animals recovery occurred after removal of 30 per cent of the blood and in the other animal after 28 per cent. These figures coincide closely with the experiment cited by Cannon (1923), in which only after 40 per cent of the blood had been lost was the adaptive ability of the vasoconstrictors surpassed.

Recovery of a normal pressure could be brought about promptly by a rapid reinjection of the removed blood. If, however, the blood pressure

was allowed to remain long at the shock level after the hemorrhage, it could not be permanently restored. When the blood pressure was restored and maintained after a prompt reinjection of the blood, the critical level

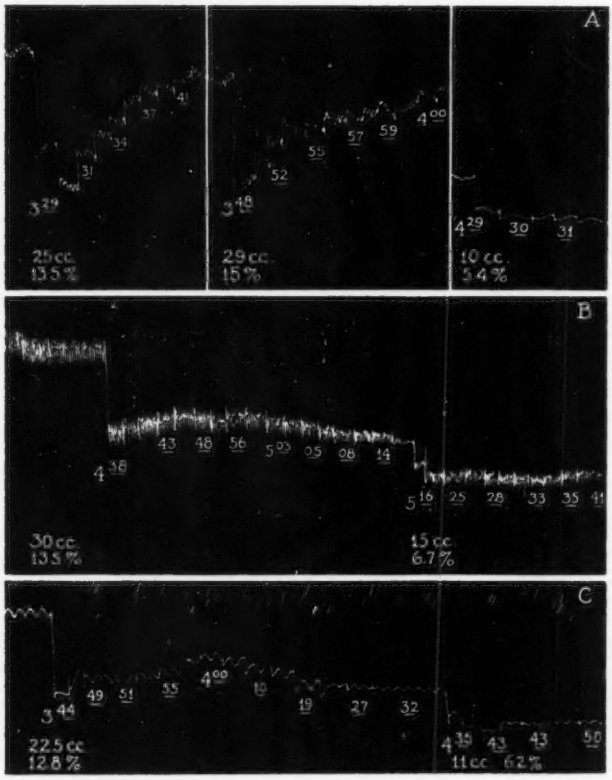

Fig. 1-a. Normal cat, "dial" anesthesia. The response of blood pressure to three hemorrhages of 13.5, 15 and 5.4 per cent of the total blood volume, respectively.

b. Cat, "dial" anesthesia, previously injected with 30 mgm. ergotamine. The responses to two hemorrhages of 13.5 and 6.7 per cent.

c. Sympathectomized cat, "dial" anesthesia. The responses to hemorrhages of 12.8 and 6.2 per cent.

was reached, in a second series of bleedings, with loss of a much smaller volume of blood than in the first series.

The response of sympathectomized animals. Erlanger, Gesell and Gasser (1919) observed that after section of the splanchnic nerves and removal of the abdominal sympathetic chains, animals went into shock much faster

than normal animals. The two animals which they described, however, survived the operation very badly and were in poor condition at the time of experiment. In the present investigation a study of the reaction to

TABLE 1

Response of normal, sympathectomized and ergotaminized cats to hemorrhage

CAT	DATE	WEIGHT	AMOUNT OF BLOOD WITHDRAWN BEFORE ANIMAL FAILS TO RECOVER	RESPONSE OF VASOCONSTRICTORS			REMARKS
				First bleeding	Second bleeding	Third bleeding	
			A. Normal animals				
		kgm.	*per cent*				
3	Feb. 11	4.3	41	Good	Good	Fair	On 4th bleeding pressure remains low
4	Feb. 12	3.7	41	Fair	Good	Good	Pressure falls after recovery from 3rd bleeding
5	Feb. 16	3.7	43	Good	Good	None	
8	Feb. 19	4.2	38	Fair	Good	None	
9	Feb. 23	3.7	31	Good	Good	None	
		Average	38				
			B. Sympathectomized animals				
88	June 30	3.1	15	Very slight	None		
94	July 1	4.4	14	None	None		
90	June 30	3.3	20	Slight, then fall	None		
96	July 1	3.7	25	Slight, then fall	None		
		Average	18				
			C. Ergotaminized animals				
6	Feb. 17	3.6	38*	Slight	Slight	Very slight	3½ mgm. ergotamine
7	Feb. 18	3.0	24*	Slight	None		9 mgm. ergotamine
10	Feb. 20	3.9	15	None	None		14 mgm. ergotamine
12	Feb. 29	4.4	13	Very slight	None		30 mgm. ergotamine
13	March 1	4.3	13	Very slight	None		40 mgm. ergotamine
		Average	14				

* Dilators to the pupil not paralyzed; figures not included in average.

hemorrhage was made on four animals which were completely deprived of sympathetic vascular control. At the time of the experiments these sympathectomized cats had been for several months in the laboratory, had gained

considerably in weight, and were in excellent physical condition. The usual blood pressure of these animals under dial anesthesia was somewhat less than that of normal animals under the same conditions, being 114, 116, 104 and 174 mm. Hg as compared with the average value of 149 mm. Hg in six normal animals. The method of bleeding the sympathectomized animals was the same as that described above.

It was found that sympathectomized cats as compared with the normal were extremely sensitive to hemorrhage (see fig. 1-c, table 1-B). Removal of 12 to 15 per cent of the blood was followed by a drop in pressure from which the animals never recovered. The degree of fall immediately after the hemorrhage was about the same in the two groups, but in the subsequent recovery phase a marked difference was noted. Whereas in normal animals, after the first bleeding the blood pressure progressively and promptly returned to almost its former level, in sympathectomized animals the compensatory adjustment was in some cases entirely absent or in others very slight. In the latter group five or ten minutes after the initial fall of blood pressure there was a slight recovery of pressure which, however, never nearly approximated the previous level and fell again within a few minutes to the original point reached just after the hemorrhage (see fig. 1-c). This slight transitory compensatory response was never observed in sympathectomized animals after the second or any subsequent hemorrhage. After each bleeding the pressure fell further and either remained at a constant low level or progressively decreased. The pressure in the sympathectomized animals could in some cases be restored to normal by reinjection of the total volume of blood removed, but this level was maintained for only a short time, a progressive slow fall taking place. No recovery was seen if a small hemorrhage was caused after the reinjection of the blood.

The response of ergotaminized animals. Dale (1906) first showed that ergotoxine when administered in suitable doses paralyzes the peripheral vasoconstrictors, whereas in smaller doses it acts as a stimulant to these nerves. Since that time numerous investigators have studied the action of ergotamine on sympathetic nerves and its effect on vasomotor reflexes. Heymans and Régniers (1927) observed that ergotamine depresses or abolishes the action of vasoconstrictors in the cervical sympathetic distribution and also suppresses the vasomotor reflexes arising in the carotid sinus. Paralysis of vasomotor phenomena in the splanchnic area, vagina and nasal mucosa, and abolition of pressor reflexes produced by stimulation of the peroneal or popliteal nerve were also produced by means of ergotamine (Van Dyke, 1926; Wright, 1930). Different parts of the sympathetic system are known to have different degrees of sensitivity to ergotamine. According to Anrep (1922) the secretory fibers in the salivary gland are more sensitive to the drug than the vasoconstrictors, and MacKay (1929)

observed that although the secretory nerves are paralyzed before the vasoconstrictors, both sets of nerves cease to act before the sympathetic dilators of the pupil cease. Some difference of opinion exists regarding the dose of ergotamine necessary to paralyze the vasoconstrictor nerves. Freeman (1933) found that under dial anesthesia larger quantities are required to reverse the action of adrenalin than under ether. Different investigators have used doses of ergotamine varying from 0.025 mgm. up to 4 or 5 mgm. per kgm. for partially inactivating or paralyzing sympathetic fibers.

The ergotamine used in the majority of our experiments was the Burroughs and Wellcome preparation, although in a few cases the Sandoz preparation was employed. Since complete paralysis of the vasoconstrictor mechanism was desired, large doses were used (5.10 mgm. per kgm.), such as were employed by Smith (1928) to obtain reversal to adrenalin in dogs under ether. Owing to the difficulty in the present investigation of obtaining uniform adrenalin reversal after ergotamine, abolition of the dilating effect on the pupil when the cervical sympathetic nerve was stimulated with a strong current (coil 5–7) was taken as the index of paralysis. This index seemed reasonable because the dilator fibers to the pupil are known to be more refractory to ergotamine than are many other sympathetic nerves, vasoconstrictors included, and their paralysis, therefore, would produce an animal resembling one which was completely sympathectomized. The ergotamine was injected intravenously at a very slow rate and the sympathetic nerve in the neck was stimulated every few minutes. Immediately after the start of the injection there was a pronounced rise in blood pressure and an acceleration of the heart. The pressure remained high for some minutes and then gradually started to fall and continued to do so until the injection was stopped. In all experiments the injection was discontinued before the pressure had fallen below its level at the start of injection. Usually when this point was reached the dilators to the pupil did not respond to sympathetic stimulation. In order to have a normal blood pressure after large doses of ergotamine it was necessary to introduce artificial respiration at the beginning of the experiment and to proceed with the injection very slowly.

After paralysis of the sympathetic nervous system with ergotamine the animal was bled in the way previously described. The initial fall of pressure after the first hemorrhage of 12 to 15 per cent was similar to that in normal animals but there the resemblance stopped. As in the sympathectomized animals the recovery phase was either very slight or entirely lacking. When it was present at all it was greatly delayed in its appearance and of short duration, the blood pressure soon going back to its previous low level (see fig. 1-b). Further bleedings only resulted in a still greater lowering of the pressure, and no indication of a recovery was ever seen. As compared with sympathectomized cats the ergotaminized ani-

mals seemed to be even more sensitive to hemorrhage in that their ability to recover after the first bleeding was even less marked. It is clear that if sufficient ergotamine is given the pressure reaches the "breaking point," from which there is no recovery, at the first hemorrhage of 12 to 15 per cent (see table 1-C). If smaller doses of ergotamine are given and the dilators to the pupil are not affected, the animals show a slight compensatory reaction to hemorrhage which is much less than that shown by a normal animal.

Several experiments were performed in which a comparison of the compensatory reaction to hemorrhage before and after ergotamine was attempted in the same animal. The animal was first bled in the usual way until the compensatory mechanism failed to act. The warm heparinized blood was then reinjected and when the pressure was restored and had been maintained at its normal level for some time, ergotamine was given and the animal again bled. In every case after ergotamine the compensatory mechanism did not react to the hemorrhage. This type of experiment, however, does not offer first rate evidence, because control experiments showed that bleeding and reinjection of the blood, even though there seems to be a good recovery, greatly increase the susceptibility to a second hemorrhage.

Hoskins (1931) suggested that it would be of interest and within the realm of possibility to express the general "vitality" of an individual quantitatively in terms of a "homeostatic index," which would be a combined expression of the ability to react to a large number of distorting influences. The present investigation may be considered to a certain extent from this point of view, in that an attempt has been made to determine the relative efficiency of some of the stabilizing influences which come into action in the maintenance of homeostasis in normal animals and in those which have been deprived of sympathetic impulses, both by the removal of the sympathetic nervous system and by its paralysis with ergotamine.

The three groups of animals described in this and the foregoing papers were subjected to a number of standard test conditions which were sufficiently severe to produce measurable variations in the normals. By the use of such tests of the ability to make adjustment to high and low temperatures, low oxygen, reduction of blood sugar and hemorrhage, it was found that inactivation of the sympathetic system rendered the animals much more sensitive to the distorting influence. Thus, on exposure to heat the average hyperthermia occurring in ergotaminized animals was twice as great as that in the normal, while the rise of temperature in sympathectomized cats was only slightly less than this. This decreased capacity to regulate the body temperature in heat is doubtless largely due to lack of vasomotor control and the resulting peripheral dilatation which normally occurs. Similarly, on exposure to cold the mechanisms preventing a

fall of body temperature were relatively inefficient in sympathectomized and ergotaminized as compared with normal animals, only in this case, in contrast to the response to heat, the greater incapacity existed in the sympathectomized cats. A comparison of the ability to retain consciousness in an atmosphere low in oxygen showed that the normal animals could resist at least twice as long, without any ill effects, the standard test condition. Also, in the response to a small dose of insulin great differences in the occurrence of marked hypoglycemic symptoms and convulsions were seen—sympathectomized animals were most susceptible and ergotaminized only slightly less so. In the reaction to hemorrhage normal animals could adjust themselves to loss of about twice as great a volume of blood as could sympathectomized, and in this instance the ergotaminized cats were even more susceptible to hemorrhage than the sympathectomized. The information obtained from this study lends further support to the principle formulated by Cannon (1929) that the sympathetic nervous system is of major importance in maintaining certain homeostatic conditions.

SUMMARY

1. Normal, sympathectomized and ergotaminized animals were compared in their ability to recover from successive hemorrhages.

2. From 30 to 45 per cent of the blood can be removed from normal cats in 3 or 4 bleedings before the compensatory vasoconstrictor mechanism fails to raise the pressure (see fig. 1-a and table 1-A).

3. Sympathectomized animals are much more sensitive to hemorrhage than are normal animals. The compensatory reaction to a single removal of 13 to 15 per cent of the total blood volume is in some cases absent and in other cases very slight (see fig. 1-c and table 1-B).

4. Animals with the sympathetic system paralyzed by ergotamine closely resemble sympathectomized cats in their inability to withstand hemorrhage, but in most cases they show an even greater lack of vasoconstrictor compensation (see fig. 1-b and table 1-C).

We wish to express our appreciation to Dr. W. B. Cannon for suggestion of the problem described in the above and previous three papers and for his kindly interest throughout the course of the work.

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HYPOPHYSECTOMY IN PREGNANT RABBITS[1]

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In view of the intimate relationship between the pituitary body and pregnancy, it is surprising that so few attempts have been made to study the effects of removing this gland at various periods of gestation. Aschner (1912) recorded the results of hypophysectomy upon three pregnant dogs. There is no mention of post-mortem microscopic examination nor of any control operations.

Fee and Parkes (1929) performed a few experiments on rabbits which were designed to show the effect on ovulation of removing the anterior pituitary at different intervals after copulation. The interval between coitus and the rupture of the follicle in this species was known to be between ten and fourteen hours. Whether or not the stimulus resulting from coitus acts directly on the ovary or indirectly through the pituitary body was to be shown by Fee and Parkes' experiments. Their operative procedure consisted in partial decerebration in order to lift out the hypophysis. The animals were kept under continuous narcosis and survived about twenty-four hours. Their experiments were adequately controlled and showed that hypophysectomy within the first hour after copulation prevents the rupture of the follicle for at least sixteen hours, whereas hypophysectomy later than the first hour post coitum has no apparent effect on the time of ovulation. In a subsequent publication Deanesly, Fee and Parkes (1930) stated that with the technique just mentioned they had been able to observe the formation of the corpus luteum up to thirty-six hours post coitum. They found that the development of the corpus luteum in decerebrated hypophysectomized and in decerebrated non-hypophysectomized rabbits was similar to but slower than in normal healthy animals.

The next publication on this subject was a short paper by Pencharz and Long (1931). These observers used rats and found that when hypophysectomized between the eleventh and twentieth days of pregnancy the period of gestation was lengthened by three or four days, and then terminated in the death of the mother. This outcome was seemingly due to resorption of dead fetuses, and in one rat the removal of the uterine contents prevented the death of the mother.

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In 1931 Smith and White published some results of their researches on this problem. These investigators used an oral approach and were able to hypophysectomize rabbits so that the animals recovered fully from the operation. They confirmed the completeness of the removal of the gland by examining serial sections of the contents of the sella. Their operations required more than an hour to perform, and consequently they could not confirm the observations of Fee and Parkes that withdrawal of the anterior pituitary body within one hour post coitum prevents ovulation. They were, however, able to corroborate the finding that ovulation occurs normally in rabbits if the hypophysis is removed later than one hour after copulation. The fact that Smith and White had healthy animals living indefinitely after the operation permitted them to study the effects of hypophysectomy on the development of the corpus luteum. They confirmed the work of Deanesly, Fee and Parkes and found that for the first forty-eight hours after hypophysectomy the corpora lutea appeared to develop normally. Ovaries of hypophysectomized rabbits examined on the fourth and eighth days after operation showed that little or no growth had occurred in the corpora after the second day. One rabbit was pregnant for twenty-two days when hypophysectomized. Four days later the uteri contained three dead fetuses. The corpora lutea showed regression and avascularity, which observation suggests that a secretion of the anterior pituitary is essential for functional maintenance of the corpus. Recently Allan and Wiles have given a preliminary account of the effect of hypophysectomy on pregnant cats. Still more recently White has recorded that blocking of the pituitary circulation causes termination of pregnancy in rabbits. He reports also the results of hypophysectomy on five rabbits during the latter part of pregnancy, and finds that abortion occurs on the second and third days after operation. These findings are in agreement with those reported in the concluding part of this paper.

The purpose of the present paper is to record the effects of hypophysectomy in rabbits at varying intervals in the period of gestation. The first set of experiments show the effect of removal of the pituitary body on ovulation and corpus luteum growth. In the second series the gland was removed on the third day after mating. The next group of experiments deal with the effects of hypophysectomy in mid-pregnancy, and the concluding series show the result of withdrawing the hypophysis from four to seven days before term. In a subsequent paper written with Dr. Samuel R. M. Reynolds a series of experiments will be reported which were designed to elucidate the mechanisms by which some of these various effects of hypophysectomy are brought about.

Throughout the experiments, efforts were made to use only postpartum does that had demonstrated their ability to deliver normal young. The operative technique consisted of an intra-orbital approach to the sella.

OPERATIVE TECHNIQUE. The exposure of the orbital fossa is facilitated by slightly elevating the animal's head and by turning the thorax and head to one side. A Bausch-Lomb optical lamp has been found to provide ideal illumination for all steps in the operation although any light of average intensity can be placed so as to illuminate the orbit adequately. The first step in the actual operative procedure is to enlarge the palpebral fissure with a lateral incision beginning just below the temporal canthus and a medial one extending 1 cm. from the nasal canthus. The flap constituting the lower lid is then retracted and the palpebral conjunctiva is divided (fig. 1). This brings into view the ligament bridging the posterior half of the zygomatic arch, part of which forms the infra-orbital ridge. By splitting and excising this ligament one is able to grasp the underlying pad of fat (the infra-orbital gland), and with gentle traction

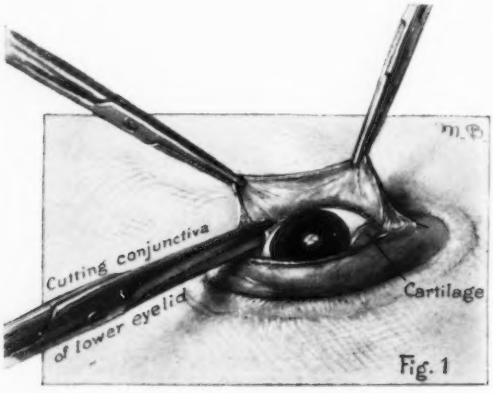
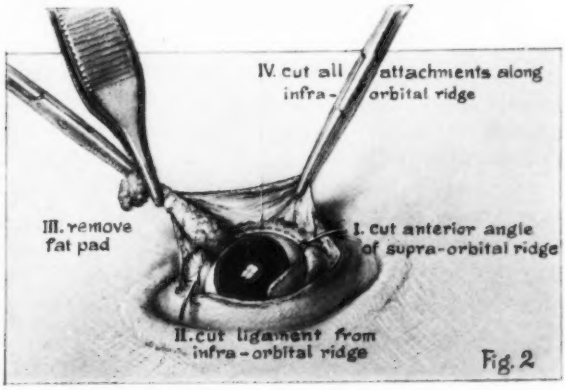


Fig. 1

this fat is withdrawn and excised. This manoeuvre gives considerable space in the orbit and facilitates the separation of all remaining attachments of the eye from the infra-orbital ridge (fig. 2). The tissues attached to the medial end of the supra-orbital process are incised so as to bring into prominence the abrupt termination of this portion of the frontal bone (fig. 2–1). This step is of importance since it delineates a landmark which is essential in the later stages of the operation. Up to this point the operative procedure has been to prepare for the anterior displacement of the eye.

In the rabbit there is an enormous venous sinus covering the posterior half of the eye. An accurate description of this sinus is given by F. A. Davis. With practice it is possible to insert a spatula carefully into the deepest part of the orbit and to bring the spatula gradually forward so as to compress the orbital sinus and to deliver the eye anteriorly without

causing the slightest bleeding (fig. 3). By properly tilting the spatula one is able to secure and to maintain the exposure that is necessary to complete the operation. If, however, the venous sinus or any tributaries are torn there will be profuse hemorrhage that can be stopped only by packing the orbital cavity with gauze for a few minutes. With the eye held out


IV. cut all attachments along infra-orbital ridge
III. remove fat pad
I. cut anterior angle of supra-orbital ridge
II. cut ligament from infra-orbital ridge
Fig. 2

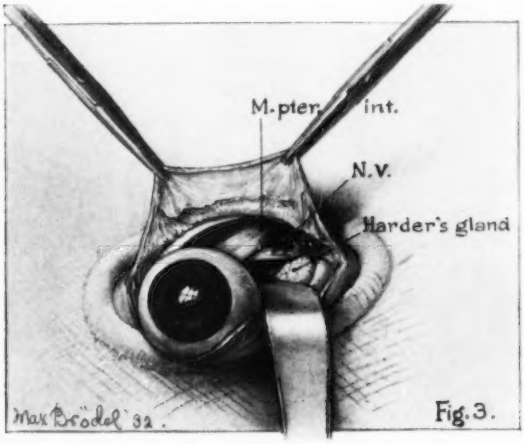


Fig. 3.

of the orbit the next step in the operation has to do with bringing into view the second important landmark. This is the point along the medial edge of the internal pterygoid muscle at which the 5th nerve emerges. This muscle is a broad flat structure which is readily seen. Covering the nerve, however, there is a layer of areolar tissue and fat, which can usually

be removed without causing bleeding. If, however, bleeding occurs, it is always venous in character and can be stopped by pressure with gauze or by shifting the tip of the spatula so as to occlude the tear in the vein. The next step in the operation is to puncture the exceedingly thin lateral wall of the sella turcica, which lies behind the internal pterygoid muscle. To do this a straight artery forceps is placed in the groove at the medial edge of the supra-orbital process and is passed directly backward along the superior border of the 5th nerve, under the edge of the pterygoid muscle, until the bony wall of the orbit is encountered (fig. 4). *Slight* additional pressure on the clamp is sufficient to break through the bone and thereby open the sella. This aperture is enlarged by opening and turning the forceps. The bleeding that results can be stopped by packing with gauze, but usually it is more practical to proceed with the operation

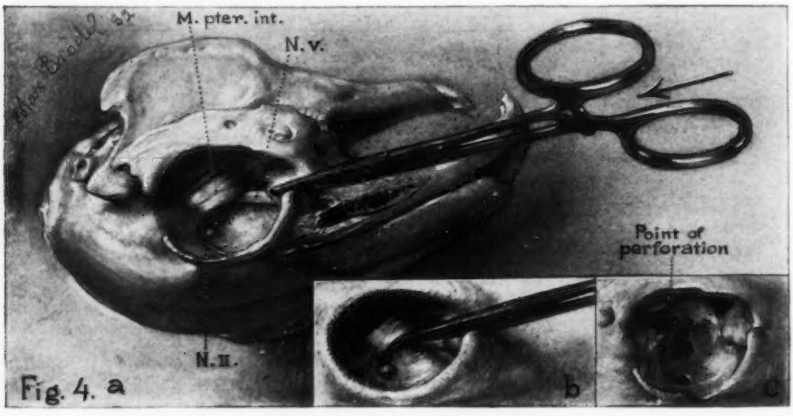
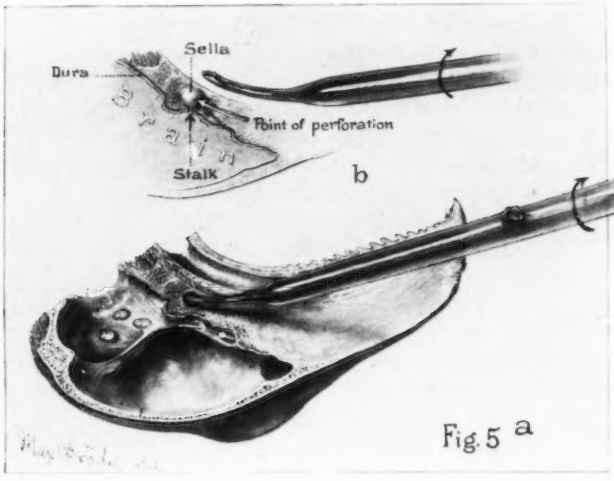


Fig. 4. a

by aspirating the blood through a tube connected with a water-suction pump. With the bleeding taken care of so that the operator's view is unobstructed, a glass aspirating tube (fig. 5) is carefully inserted into the sella and suction applied by placing one's index finger over the opening in the side of the tube. If the gland does not come out immediately the tube is rotated 50° or 60° so that the bevelled tip dislodges the hypophysis. Care must be taken when placing the aspirating tube into the sella to be certain that the bevel faces away from the infundibulum. Unless this precaution is taken the suction may be applied to the base of the brain and cause sufficient injury there to kill the animal. As soon as the sella is empty the aspirating tube is withdrawn, and the spatula is lifted away and the eye replaced in the orbit. Gentle pressure on the globe serves to stop quickly any bleeding that persists. Each incision is closed with a single suture of fine silk. No dressing is applied to the eye. In no cases

has there been the slightest evidence of postoperative intracranial infection. With experience the time required to perform the operation has been reduced from an average of twenty-five minutes to one of less than fifteen minutes. Many of the recent operations have been completed in ten minutes.

One further word concerning the landmarks is needed. Recently we have found in the small Black Dutch variety of rabbits that it is necessary to pass the tip of the perforating artery forceps along the lateral border of the 5th nerve and not along the superior margin. It seems advisable to use always the same variety of rabbits for hypophysectomy and to familiarize oneself thoroughly with the anatomical relationships of that variety before attempting the technique described in this communication.



In each series of experiments adequate control operations were carried out, as will be described in the text. All operations, whether laparotomies or hypophysectomies, were done under ether anesthesia given by intratracheal insufflation. Post-mortem examination was made of the sella of each animal, and in all but eight instances serial sections were made of the sella or of its contents so as to verify the complete absence of hypophyseal tissue. After the first twenty sets of serial sections were examined it was obvious that in pregnant rabbits minute remnants of anterior lobe cells are incapable of preventing a physiological deficiency which is identical with that observed in totally hypophysectomized rabbits. The eight animals whose sellae were not sectioned serially were ones in which the sellae were obviously empty at autopsy and the post-operative behavior

of the animals had been in such perfect agreement with other totally hypophysectomized animals that serial sections were superfluous.

The post-coitum operations. This series of operations consists of eight rabbits in which the hypophysis was removed at intervals varying from twenty-six minutes to three hours after mating. In five of these eight the gland was removed within thirty-five minutes after coitus. The ovaries of these rabbits were examined on the second and third days after the hypophysectomy and no sites of ovulation nor young corpora lutea were seen. On histological examination the ovaries seem quite normal. In three of these animals injections of urine of pregnancy were given on the day of the exploratory laparotomy and within forty-eight hours another examination was made. In each case the ovaries contained many recently ovulated follicles, thus showing that follicles capable of ovulation were present, and also indicating that urine of pregnancy can produce ovulation in the absence of the hypophysis. In the remaining three rabbits in this series of eight the intervals between mating and the actual withdrawal of the gland were fifty minutes, two and a half hours, and three hours. These three animals ovulated and had one ovary removed on the third or fourth post-operative day. Each of the removed ovaries showed corpora lutea which had not progressed as far as those of the control animals, but these alterations are not so marked as expected. There is less vascularity, and the cells are not so compact nor so full as in the controls. From seven to ten days later the remaining ovary was removed, and each showed obvious and extensive atresia. In some cases the sites of the old corpora could not be seen macroscopically. Serial sections of these regressed ovaries removed seven and ten days after hypophysectomy show that the ovaries are in a quiescent state. Furthermore, at the second laparotomy there were extensive changes in the uteri, which were found to be thin, pale, atonic, and friable. Histologically these regressive changes are confirmed. There is no distinct evidence of oestrogenic growth nor of progestinal proliferation.

To make certain that the anesthesia and the trauma of the operation were not responsible for the results obtained, eight rabbits were treated similarly to the preceding group except that the hypophysis was not withdrawn. Care was taken to puncture the sella and to see that the amount of blood lost was greater than in ordinary operations. In every one of these eight rabbits ovulation occurred, and in seven of the eight a normal pregnancy followed. The exceptional rabbit which did not become pregnant was one in which the exploratory laparotomy and oöphorectomy was performed twenty-four hours after coitus instead of on the third post-operative day, as was done with the other rabbits. Whether or not this operative procedure interfered with the migration of the ova cannot be definitely asserted, but the explanation seems plausible.

The three-day series. The second group of eleven rabbits were those in which the hypophysis was removed on the third day after coitus. In all of these animals a preliminary laparotomy was carried out to make certain that normal three-day corpora lutea were present. The hypophysis was then removed, and after intervals varying from four to ten days a second laparotomy was performed. In every instance at the second operation the corpora were seen to have regressed and the ovaries to have atrophied (fig. 6a and 6b). No pregnancies had occurred and the uteri were involuted. In two rabbits an effort to produce placentomata was made. Neither attempt was successful, but one cannot attribute this failure to a refractory uterus since the operator had never before attempted to produce placentomata. Sections of ovaries removed from four to ten days after hypophysectomy show progressive alterations. The corpora lutea become steadily smaller (figs. 7 and 8). The lutein cells become vacuolated and pale. The vascularity

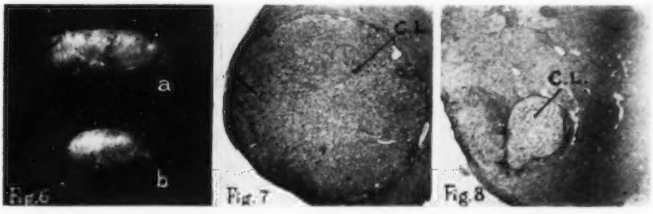


Fig. 6A. Ovary, removed 3 days post coitum.

6B. Ovary from same animal removed 10 days post coitum and 7 days after hypophysectomy.

Figs. 7 and 8. Sections of corpora lutea from 6A and 6B respectively. Both × 25.

of the corpora is diminished and the amount of connective tissue is relatively increased.

As controls for these eleven animals four others were subjected to laparotomies and intra-orbital operations in which the sella was punctured but the hypophysis was not withdrawn. In three of these the amount of blood lost during the operation was measured and shown to be appreciably more than the quantity lost when complete hypophysectomy was carried out. Each of the four rabbits became pregnant and delivered litters of healthy young. The periods of gestation lasted from thirty to thirty-three days.

The mid-pregnancy series. The rabbits operated on in mid-pregnancy—that is to say, on the fourteenth day post coitum—numbered sixteen, in eleven of which the hypophysis was removed and five of which were operative controls. The diagnosis of pregnancy was made by palpation after general anesthesia had been induced. In those cases, however, where there was any doubt as to the existence of pregnancy, the abdomen

was opened for confirmation. Inasmuch as all the rabbits were mated only once and were kept in separate cages, the duration of pregnancy was fairly accurately known. None of the eleven hypophysectomized rabbits delivered living young. Three of the group resorbed most of the uterine contents within a week after the operation. One died on the sixth and another on the ninth post-operative day. At autopsy it was seen that their uteri were filled with necrotic, partially absorbed placentae. Four rabbits passed fetal fragments and blood clots from the fifth to the seventeenth post-operative day. In the last two rabbits in this series laparotomies were performed six, twelve, twenty-four, thirty-six, and forty-eight hours after hypophysectomy in order to determine the time at which the fetuses died. At each laparotomy one gravid segment of uterus was excised and both fetus and placenta were examined. Dr. W. B. Vandegrift assisted at these operations and suggested that spontaneous beating of the fetal heart is an accurate criterion of viability. We found that in one instance the fetus was detached and dead twenty-four hours after hypophysectomy. By thirty-six hours other fetuses had become edematous and distorted, and twelve hours later the remaining fetuses were necrotic. In the second rabbit examined periodically the hypophysis was not totally removed but similar changes were observed to occur. The only difference between these two animals was that the death of the fetuses occurred twelve hours later in the partially hypophysectomized rabbit. The argument that closely approximated anesthesias and many laparotomies affected the results is not valid since we were able to recover from a non-hypophysectomized rabbit living fetuses six, twelve, twenty-four, thirty-six, and forty-eight hours after an intra-orbital puncture of the sella. The time of the death of the fetus is not, however, the most interesting observation which resulted from the frequent examination of the pregnant uteri because histological study of the placentae shows unexpected expansion of the trophoblast. The apparent overgrowth of this tissue can be seen from gross inspection of the organ and appears within twelve hours after the removal of the hypophysis. The trophoblastic hypertrophy reaches its maximum in the twenty-four specimen. Dr. W. G. MacCallum and Dr. Emil Novak have looked over this material and confirm the observation that there is apparent hyperplasia of the trophoblast. During the second twenty-four hours after hypophysectomy degenerative changes in the placenta are recognizable. It is of interest to note that similar alterations cannot be seen in the placenta of a rabbit oöphorectomized on the fourteenth day of pregnancy. In this placenta there is no such gross alteration, but histologically one can see evidence of degeneration on the maternal side of the organ. A laparotomy twelve hours after bilateral oöphorectomy in mid-pregnancy showed that the fetuses were alive, whereas a second examination twelve hours later

showed that all the fetuses were dead. It seemed at this operation that the uteri were in a state of tonic contraction and that the fetuses had been squeezed to death. Twenty-four hours later the placentae and remaining fetuses were sufficiently necrotic to bring about the death of the parent. The differences noted between the oöphorectomized and the hypophysectomized rabbits will have to be confirmed many more times before one is warranted in asserting that the interruption in mid-pregnancy caused by hypophysectomy is not indirectly through the degeneration of the corpora lutea. But such a conclusion is suggested by our findings.

The five animals operated on as controls in the mid-pregnancy group gave birth to litters of healthy young on the thirtieth and thirty-first days.

The late pregnancy series. The final series of experiments were those in which the hypophysectomy was performed on the twenty-fourth, twenty-fifth, or twenty-sixth day of gestation. This group is made up of six rabbits in which the gland was completely removed, three in which it was partially removed, and nine controls. For forty-eight hours after the operation the only apparent change in the hypophysectomized rabbits was sluggishness and inactivity. After this time the rabbits passed one or more fetuses and at intervals of four or five hours passed additional fetuses. These were never macerated and all those seen at time of birth were living but died within an hour. Three of the animals quickly returned to a normal state; a fourth died on the third post-operative day after having passed several fetuses during the preceding night. At autopsy the uteri showed placental sites and one dead fetus. Two rabbits continued to pass fetal fragments on the fourth, fifth, and sixth post-operative days. None of the rabbits made nests. The three rabbits in which the gland was only partially removed gave birth on the twenty-eighth day of pregnancy (third post-operative day) to several young, which died in a few hours. These young were born at intervals of several hours. The five rabbits surviving complete hypophysectomy were placed with a male every day for a week following delivery. They refused to mate until the seventh or eighth day, and after copulation they did not ovulate. This slowness in mating is not significant as similar behavior was observed in some of the control animals. The nine controls furnish adequate proof that neither the operative approach, the anesthesia, nor the loss of blood was responsible for the results observed following hypophysectomy. These nine rabbits were operated on and the sellae were opened. Considerable bleeding was encouraged, so much so that two of the animals were expected to die from hemorrhage. The operations were on the twenty-fourth to twenty-seventh days of pregnancy. All these rabbits delivered normal litters of healthy young. The two that had the most severe hemorrhage did not give birth to their litters until the thirty-third and thirty-fourth days, whereas the others were delivered on the thirtieth and

thirty-first days. In order to compare the effect of bilateral oöphorectomy with hypophysectomy, both ovaries were removed from a rabbit on the twenty-fourth day of pregnancy. This animal began to abort twenty-two hours later, and at this time a laparotomy was performed. The fetuses were alive, the uterus was actively contracting and irritable. Within thirty minutes the remaining fetuses were born. They breathed and moved and seemed quite normal. Similar happenings were observed in a rabbit which was entirely hypophysectomized and completely oöphorectomized. In a third rabbit all the corpora lutea were removed on the twenty-fifth day of pregnancy, and abortion occurred between twelve and twenty hours later.

Discussion. The first group of experiments recorded in this paper confirm the observations of Fee and Parkes and Smith and White on the effects of hypophysectomy shortly after coitus. Our records show that the removal of the hypophysis from rabbits within thirty-five minutes after copulation prevents ovulation although follicles capable of ovulating are present. Furthermore, it is shown that urine of pregnancy can cause ovulation in the absence of the hypophysis. When the hypophysis is withdrawn from fifty minutes to three hours after mating, ovulation takes place and corpora lutea develop but pregnancy does not occur. The corpora develop for two days and then retrogress rapidly. In the rabbit in which hypophysectomy is performed three days after coitus, implantation may take place but pregnancy does not occur. Grossly no evidence of implantation was apparent four days after hypophysectomy, and at this time involution of the uteri and atrophy of the ovaries were noticeable. Within a week the ovaries and corpora lutea undergo extensive atresia, and the uteri have taken on the appearance of those of castrates. The removal of the hypophysis in mid-pregnancy in rabbits causes the death of the fetuses about the twenty-fourth hour after operation although external evidences of this fact are lacking until the fifth post-operative day. At this time absorption of the products of conception is well advanced and well tolerated in some cases, while in others the mother extrudes per vaginam macerated tissue, and in still other instances the parent dies apparently from the absorption of necrotic products of conception. Furthermore, the placenta of rabbits hypophysectomized on the fourteenth day of gestation shows within twenty-four hours extraordinary hyperplasia of the trophoblast, and within the next twenty-four hours degenerative changes appear in this organ.

The results of the removal of the hypophysis on the twenty-fourth to twenty-sixth day of gestation are not as clear cut as when the gland is withdrawn earlier in pregnancy. That the pregnancy is terminated is certain, but whether this is due to alterations in the placenta or in the corpora or to the death of the fetus is not apparent. Why some of the

animals abort living fetuses which quickly die and others absorb dead fetuses is also not clear. The experiments recorded raise doubt as to whether or not any part of the hypophysis is essential for labor.

The writer wishes to thank Dr. Samuel R. M. Reynolds for his untiring assistance and many fruitful suggestions.

CONCLUSIONS

The hypophysis is essential for ovulation and for the life of the corpus luteum in rabbits. Without the hypophysis implantation either does not occur or, if it occurs, does not persist. In rabbits the hypophysis is essential for the continuation of pregnancy. Whether or not this is due to its effect on the corpus luteum or directly on the uterine products of conception is not clear. There is some doubt as to whether or not any part of the hypophysis is essential for labor.

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MODIFICATION OF NERVE AFTER-POTENTIAL AND REFRACTORY PERIOD BY CHANGES OF IONIC ENVIRONMENT: NEW CASES OF PHYSIOLOGICAL ANTAGONISM BETWEEN UNIVALENT AND BIVALENT CATIONS[1]

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The well-known effect of the potassium ion on the demarcation potential of nerve, together with the possibility of an inverse relationship between this potential and the after-potential (Verzár, Furusawa) recommended the trial of this ion for its possible effect on the after-potential. The demonstration by Bishop that veratrine resembles potassium qualitatively in its action on the demarcation potential heightened the possibility that potassium might resemble veratrine in increasing the after-potential; but preliminary experiments at once showed that the relation is not so simple as this, since potassium has exactly the opposite action—i.e., it decreases the after-potential. When calcium on the other hand was found to produce a veratrin-like increase of after-potential, this new case of physiological antagonism between univalent and bivalent cations seemed worthy of investigation for the light it might throw on the nature of the after-potential. Accordingly the effect of these two groups of ions on this property of nerve was studied in some detail, and the study was extended to include certain other ions and certain other nerve properties.

METHODS. The apparatus and technique employed were in general the same as in other recent publications from this laboratory (12), (16). Grid leaks of 1 megohm were used in all experiments, as the duration of the after-potentials was not great enough to introduce a significant "condenser error" with these leaks. Induction shocks from coreless Harvard coils were usually used as stimuli; the use of discharges of a 0.005 mfd. condenser in certain of the experiments is described in the section on the refractory period. Unless otherwise noted, the stimuli were strong enough to stimulate all or most of the A fibers, but none of the B fibers.

The salts employed were of good C.P. grade, and were made up in isotonic concentrations. The concentration of each salt, except sodium

[1] This work was reported in part at the meeting of the American Society for Pharmacology and Experimental Therapeutics, April, 1931.

thiocyanate and sodium oxalate, was calculated from data on the freezing-point lowering (International Critical Tables); sodium thiocyanate was used in 1 per cent solution and sodium oxalate in 1.36 per cent. All solutions applied to the nerve were isotonic, the individual salt solutions being diluted with Ringer's or other isotonic solutions when it was desired to reduce the concentration of the salt in question below isotonicity. The concentration of the individual salts is indicated by the appropriate decimal fraction of I (isotonicity); the total salt in each solution therefore adds up to 1 I. For instance, the Ringer's solution used in most of the experiments is written NaCl 0.940 I, KCl 0.035 I, $CaCl_2$ 0.025 I; De Boer's potassium-poor summer frog Ringer's solution, which was substituted for this in many of the experiments in the summer months, is written NaCl 0.970 I, KCl 0.005 I, $CaCl_2$ 0.025 I; a solution containing ten times the normal amount of potassium is written NaCl 0.625 I, KCl 0.350 I, $CaCl_2$ 0.025 I, or NaCl 0.635 I, KCl 0.350 I, $CaCl_2$ 0.015 I, according to whether the potassium ion was increased at the expense of the sodium ion alone, or with proportionate decrease of the calcium ion as well. Similarly, when a fourth cation is introduced, the formula expressing the solution becomes of the form NaCl 0.750 I, KCl 0.030 I, $CaCl_2$ 0.020 I, $BaCl_2$ 0.20 I.

Since the hydrogen ion concentration is known to affect the after-potential (12), the pH of the solutions was adjusted to 7.6 in all cases except with $BeCl_2$ and $AlCl_3$, which are hydrolyzed with precipitation of the hydroxides at this hydrogen ion concentration. The solutions of these two salts were therefore as nearly neutralized as possible, and the nerve to be treated with them soaked for half an hour before the experiment in Ringer's solution of the same pH (5.6 for isotonic $BeCl_2$, 4.1 for isotonic $AlCl_3$). The solutions were not buffered, but the nerve can probably be considered to have come into equilibrium with these hydrogen ion concentrations before the Be^{++} and Al^{+++} ion were applied.

The salts tried were the chlorides of the five alkali metals and of ammonium; the iodide, fluoride, thiocyanate, sulfate and oxalate of sodium; and the chlorides of the five alkaline earths, and of aluminium. Ringer's solution in which all the Cl' was replaced by I' was also used.

The nerve (sciatic of Rana pipiens) was not usually immersed in the modified salt solution, but by means of a camel's hair brush the solution was painted on it rather freely as it lay on the electrodes in position for stimulating and recording. The solution was usually applied over the whole nerve, but was sometimes limited to the portion under the live lead and the stimulating electrodes, with precautions to prevent its spread to the dead-live junction; no conflicting differences in the results were observed. An interval of fifteen minutes to an hour, sometimes with repeated application of the solution, was allowed for the nerve to adjust to the changed salt environment.

EFFECTS OF UNIVALENT AND BIVALENT CATIONS. *The after-potential.* The power of univalent cations to reduce the after-potential is illustrated for K^+ in figures 1 A, 2 and 3 B. The activity of Rb^+ and NH_4^+ in this regard is of the same order as that of K^+, while the activity of the other three univalent cations tried, Na^+, Li^+ and Cs^+, is so slight as to be difficult to demonstrate against the tendency of the after-potential to increase under the experimental conditions. The potassium ion was the only univalent cation investigated in detail.

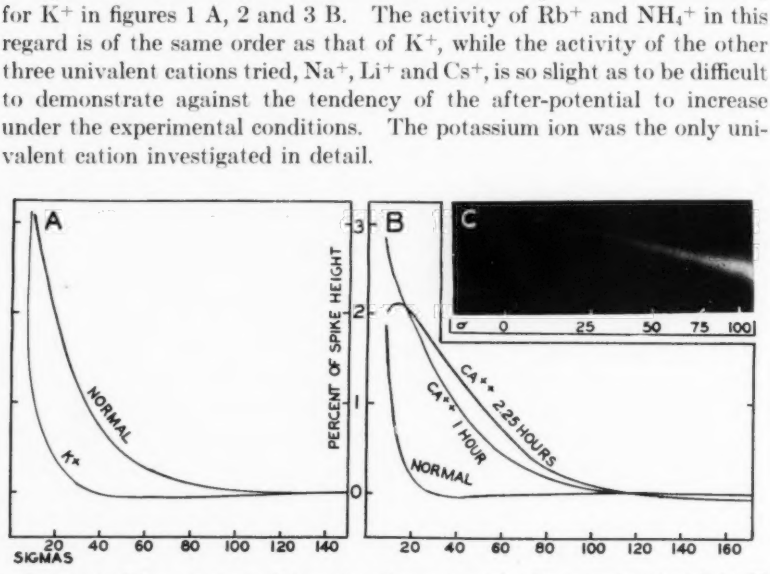

Fig. 1, A. Effect of excess K^+ on the after-potential. Nerve painted with NaCl 0.660 I, KCl 0.325 I, $CaCl_2$ 0.015 I, few minutes before record for K^+ curve taken. Maximal spike height reduced to 70 per cent of normal by this solution. The positive artifact in the normal response fell too late to appear in the figure. 7/22/31 II.

B. Effect of excess Ca^{++} on the after-potential. NaCl 0.750 I, KCl 0.030 I, $CaCl_2$ 0.220 I for times indicated. Spike height same throughout. Nerve stimulated for refractory period determinations between after-potential records. 8/6/31.

C. Effect of excess Ca^{++} on the after-potential. NaCl 0.660 I, KCl 0.020 I, $CaCl_2$ 0.320 I, 1 hour. Nerve had been stimulated as in B. Record $\times \frac{2}{3}$. 1 mm. = 15μv. Equivalent spike height = 442 mm. 10/23/31.

In the experiment reproduced in figure 1 A, the potassium concentration was nine times as great in the solution tested as in the Ringer's solution used as normal; similar effects can be obtained by increases as small as three times the original concentration. The potassium after-potential generally has the smooth decremental form seen in the figure, and usually crosses the zero potential line at 20–50σ, to be succeeded as in normal nerve by the positive artifact. This is the form and duration of after-potential seen at room temperature in fresh nerves from summer frogs (12). Further shortening of the potential to 6–8σ would be of great theoretical interest, because there should then remain only spike-potential (13); but such shortening can not be observed in potassium experiments owing to the

toxicity of the ion for the spike-potential. The crest of the after-potential is not visible after potassium treatment, except when it has been accentuated previously by veratrine or a bivalent cation. Potassium then frequently produces a form similar to the one shown in figure 2, *5* and *6*, with a distinct after-potential crest, and a much longer duration than usual after potassium. This greater duration of the potassium after-potential is occasionally seen without previous treatment with an after-potential-increasing agent. The maximum magnitude of the potassium after-potential varies widely not only with the concentration and the interval after application of the potassium-rich solution, as expected, but also with the pre-potassium magnitude of the after-potential.

The effect of bivalent cations on the after-potential is illustrated in figure 1 B and C for calcium in concentrations nine and thirteen times its concentration in the laboratory Ringer's solution. Smaller increases than this are ineffective. Mg^{++} seems to be about as active as Ca^{++}, and Ba^{++} is somewhat more active (figs. 2 and 3), while the activity of Sr^{++} has been demonstrated only after previous treatment with excess of a univalent cation, and Be^{++} has not been found to have any activity.

The increases of after-potential produced by the active bivalent cations are similar in kind to those produced by veratrine (16), though less in degree. The after-potential usually does not rise above 4 per cent of the spike potential with Ca^{++} (it may be somewhat higher with Ba^{++}), and its duration is not greater than 200–300σ even after stimulation. The crest of the after-potential is frequently visible in the responses of nerves treated with these ions (figs. 1 C; 2, *3* and *7*; 3 A *4*), and generally falls between 10 and 20σ after the stimulus; it has not been observed later than 30σ as compared with the 50–80σ maximum interval observed in veratrinized nerves. As in veratrinized nerves, the crest of the after-potential tends to fall later as the experiment proceeds (curves 2 and 3, fig. 1 B), but in contrast to veratrinized nerves, the nerves exposed to an excess of bivalent cations do not show a marked reversible delay of the crest after a period of rapid stimulation; if such delay occurs, it must be reversed so rapidly as to escape notice.

In most of the experiments chosen for reproduction, when the concentration of the potassium ion or of the calcium ion was increased, that of the other was decreased; and under these circumstances it seems possible that either one or the other of the ions is the sole active agent. Since the same effects were obtained, however, when the concentration of one ion was increased without decreasing that of the other, i.e., at the expense of the sodium ion only, it is obvious that both the potassium and calcium ions possess activity, and that the activity of one opposes that of the other. If further evidence be needed for the balanced action of the potassium and calcium ions, it may be found in the fact that substitution of sodium for either of them results in the picture produced by excess of the other.

It follows from this balanced action that while the effect of an excess of either potassium or calcium, or more generally speaking, an excess of either a univalent or bivalent cation, may be reversed by washing with ordinary Ringer's solution, it may be reversed with much greater speed and completeness by washing with an excess of an ion of the antagonistic group. The after-potential may thus be made alternately large and small at will

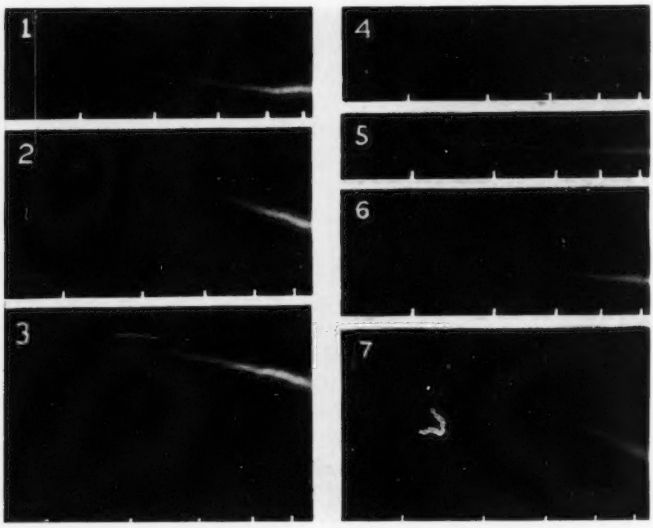

Fig. 2. Alternation of excess of bivalent and univalent cations. Records × $\frac{2}{3}$. Ordinates, 1 mm. = 7.5 μv; abscissae, time in 25σ intervals. After-potentials following responses of $\frac{1}{2}$–$\frac{2}{3}$ maximal height, except *6*. *1*, normal; equivalent spike height, 431 mm. *2* and *3*, NaCl 0.75 I, KCl 0.03 I, $CaCl_2$ 0.02 I, $BaCl_2$ 0.20 I for $\frac{1}{2}$ hour and 1 hour respectively, with intervening stimulation; equivalent spike height, 680 mm. *4*, NaCl 0.75 I, KCl 0.23 I, $CaCl_2$ 0.02 I for 1 hour; equivalent spike height 680 mm. *5*, NaCl 0.660 I, KCl 0.325 I, $CaCl_2$ 0.015 I for 1 hour; equivalent spike height 159 mm. *6*, as in 5, but maximal response; equivalent spike height 465 mm. *7*, NaCl 0.75 I, KCl 0.03 I, $CaCl_2$ 0.02 I, $BaCl_2$ 0.20 I for 50 minutes; equivalent spike height 1020 mm. 4/20/30.

(fig. 2); and as the cycle is gone through a second or third time, the change seems to be more easily brought about than in fresh nerve; it is as if some buffering mechanism of the nerve had been used up. The only exception to this ease of reversibility has been referred to above, namely, the occasional persistence of a long duration of the after-potential under potassium.

As would be expected from the results in normal and veratrinized nerve, the after-potential in nerves treated with excess of either univalent or bival-

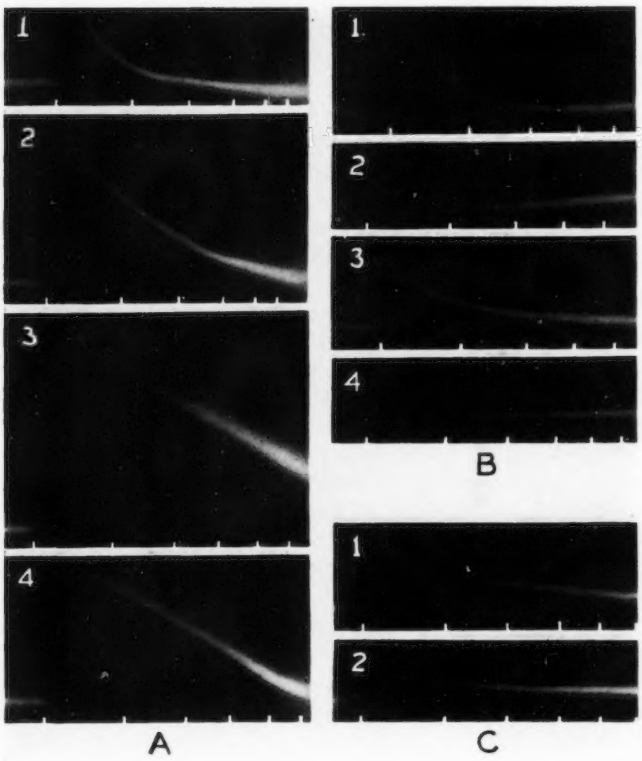

Fig. 3, A. Effect of stimulation following excess of bivalent cations. After-potential records × $\frac{2}{3}$. Abscissae, time in 25σ intervals. *1*, normal; equivalent spike height 386 mm. *2*, NaCl 0.75 I, KCl 0.03 I, $CaCl_2$ 0.02 I, $BaCl_2$ 0.20 I for 40 minutes; spike height unchanged. *3*, after ten minutes' stimulation, 120 maximal induction shocks per second; equivalent spike height 363 mm. *4*, after 45 minutes' rest; spike height still 363 mm. 5/29/31.

B. Effect of stimulation following excess of univalent cations. After-potential records × $\frac{2}{3}$. Abscissae, time in 50σ intervals. *1*, normal; equivalent spike height, 329 mm. *2*, NaCl 0.640 I, KCl 0.335 I, $CaCl_2$ 0.025 I for 35 minutes; equivalent spike height 135 mm. *3*, after 10 minutes' stimulation, 120 maximal induction shocks per second; spike height now 125 mm. *4*, after 20 minutes' rest; spike height still 125 mm. 3/30/31 II.

C. Effect of cooling barium-treated nerve. After-potential records × $\frac{2}{3}$. Abscissae, time in 50σ intervals. *1*, NaCl 0.75 I, KCl 0.03 I, $CaCl_2$ 0.02 I, $BaCl_2$ 0.20 I applied 1 hour earlier; temperature 21°. *2*, after lowering temperature of nerve chamber to 7° by introduction of ice. Removal of the ice and return of the temperature to 21° restored the form of after-potential seen in *1*. 3/20/31 II.

ent cations is greatly increased following a period of rapid stimulation, and this increase is reversed by rest (fig. 3, A and B). The usual decrease on cooling may also be observed in nerves treated with bivalent cations (fig. 3 C); nerves in which the after-potential has been decreased by univalent cations can hardly be investigated for this decrease, since the prolongation of the spike at the lower temperature tends to obscure any shortening of the already abbreviated after-potential.

The spike potential. It may be shown for the ionic alterants of after-potential, as for veratrine, that they in no way modify the time-relations of the axon spike potential up to the moment when fusion with the after-potential begins to complicate the picture: records of conducted threshold spikes (usually less than 0.5 per cent of maximum spike height, amplified to 1–4 cm. for recording) show complete identity of form before and after treatment with any of these ions. Because the univalent ions produce a form of potential in which the spike and the after-potential are inextricably blended, the behavior of the end of the spike (13) can not be studied under the influence of excess of this group. The bivalent cations on the other hand by delaying the crest of the after-potential tend to bring out the end of the spike, and the "notch" marking this varies in position as would be expected with the changes in form of the after-potential, and always falls within the normal range. For instance in figure 1 C, it may be seen at 5.5σ. Bivalent cations in excess therefore do not modify the time-relations of any part of the axon spike potential; and the same statement may presumably be made for univalent cations, in view of the experimental proof that they do not affect the time-relations of the high-potential portion of the spike.

The height of the spike potential is however greatly affected by excess of univalent cations. The height of the maximal response tends to fall fairly promptly though, except with high concentrations of these ions, not for some minutes after the decrease of the after-potential. By the time the after-potential has been shortened to $20–50\sigma$, the spike height may be anything from 100 down to 30 per cent of its normal value, and further potassium treatment extinguishes the response very promptly. If the nerve be not too much injured, the application of calcium in excess will at once restore part or all of the spike potential, and this restoration will precede that of the after-potential. When applied to normal nerve the bivalent cations neither increase nor markedly reduce the spike height; in fact, this may remain unchanged throughout a prolonged experiment with excess of the alkaline earth ions (fig. 1 B). This lesser toxicity of the bivalent cations is of course not a new observation.

The form of the spike potential will show secondary changes from at least two other causes: 1, the degree of diphasicity increases with time and probably with excess of bivalent cations, while it decreases with excess of univalent cations (14); and 2, excess of either univalent or bivalent cations

interferes with conduction, and therefore increases temporal dispersion and the duration of the conducted spike recorded after stimulation of any large fraction of the A fibers. This second effect probably explains the increase in rising-time observed by Rosenberg and Kitayama with excess of potassium and calcium. Such changes of course do not indicate any change whatever in the axon spike potential.

Irritability. The changes of nerve irritability observed in these experiments need not be set forth at length as they agree with those reported by the workers who have made detailed studies of this property (21), (5), (19), (24). The threshold to the induction shock and the condenser discharge used as stimuli was raised by an excess of bivalent cations, and was at first lowered and then raised by an excess of univalent cations.

The refractory period. The recovery of irritability after a maximal response was tested by means of an induction shock or a condenser discharge; in the first case, the resistance in the primary circuit was used to measure the variation in threshold; in the second case, the change in the voltage applied to the condenser served the same purpose. The two forms of testing shock probably had about the same effective length (0.06σ), and they gave the same results. In part of the experiments, the conditioning shock was applied at the same electrodes as the testing shock; in others, it was applied at another pair of electrodes 1 to 2 cm. farther from the leads. A few measurements of the latter part of the refractory period were also made by following the changes in the height of maximum response at various intervals after the conditioning shock; the modifications of relative refractoriness thus observed were the same as when the recovery of irritability was followed. The relatively refractory periods observed in normal nerves were somewhat shorter than those recorded in other cases at comparable temperatures, although the durations of the absolutely refractory period agreed with published data (11). The cause of the deviation of the relatively refractory period is not apparent.

With nerves subjected to an excess of K^+, the absolutely refractory period always shows some prolongation (43 per cent in fig. 4 A, 57 per cent in 4 C). Moreover, the recovery curve rises more gradually than the normal recovery curve; 80 per cent recovery of irritability thus takes perhaps twice as long in the potassium-treated nerve as in the normal (fig. 4B), while complete recovery, which is approached asymptotically and therefore occurs after an indeterminable time, is still more delayed. The changes are in part reversed by returning the nerve to Ringer's solution, but as would be expected from the general tendency of refractoriness to prolong as time goes on, the reversal is not complete.

With calcium-treated nerves, the absolutely refractory period is unchanged or possibly slightly prolonged, if the second stimulus is applied at a different point from the first (fig. 4 D). The recovery curve rises more

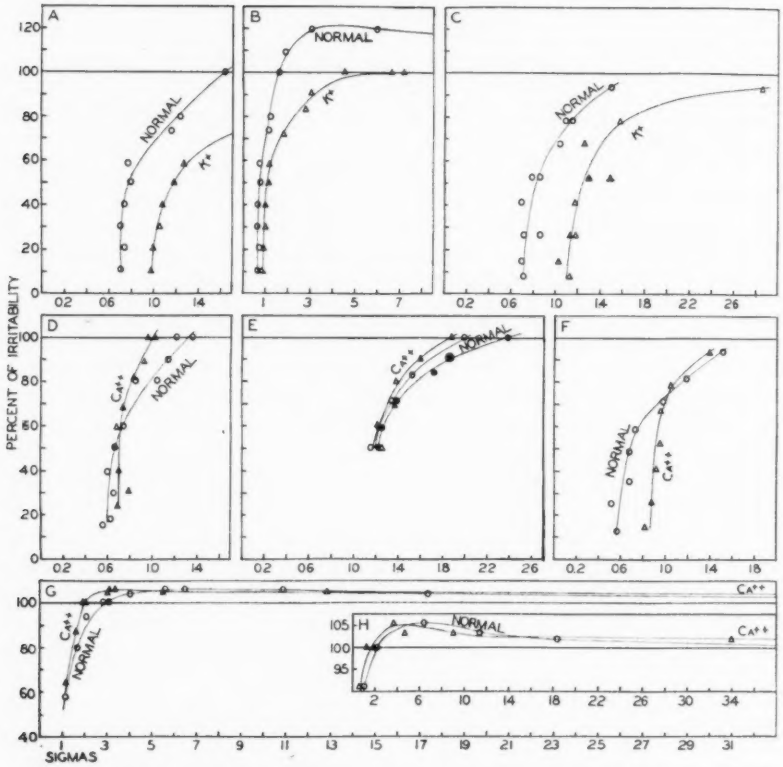

Fig. 4. Effect of cations on the refractory and supernormal periods. *A* and *B*, effect of NaCl 0.85 I, KCl 0.13 I, $CaCl_2$ 0.02 I for 40 minutes. Conditioning and testing shocks applied 1 cm. apart. Temperature 24°C. 10/28/31. *C*, effect of NaCl 0.78 I, KCl 0.20 I, $CaCl_2$ 0.02 I for 10 minutes; Ringer's solution used for normal was NaCl 0.970 I, KCl 0.005 I, $CaCl_2$ 0.025 I. Conditioning and testing shocks applied at same point. Temperature 25°C. 8/14/31. *D*, effect of NaCl 0.626 I, KCl 0.024 I, $CaCl_2$ 0.350 I for 40 minutes. Conditioning and testing shocks applied 1 cm. apart. 10/23/31. *E*, effect of NaCl 0.63 I, KCl 0.02 I, $CaCl_2$ 0.35 I for 1 hour. ○, refractory period in Ringer's solution preceding application of the calcium-rich solution; ●, refractory period $1\frac{1}{4}$ hours after painting with Ringer's solution subsequent to calcium treatment. Conditioning and testing shocks applied 18 mm. apart. Temperature 22.5°C. 11/2/31. *F*, effect of NaCl 0.647 I, KCl 0.003 I, $CaCl_2$ 0.350 I for 40 minutes; Ringer's solution used for normal same as in *C*. Conditioning and testing shocks applied at same point. Temperature 26°C. 8/13/31 II. G, effect of NaCl 0.660 I, KCl 0.020 I, $CaCl_2$ 0.320 I for 75 minutes. Conditioning and testing shocks applied 11.5 mm. apart. Temperature 22°C. 11/25/32. H, effect of NaCl 0.660 I, KCl 0.020 I, $CaCl_2$ 0.320 I for 30 minutes. Conditioning and testing shocks applied 11 mm. apart. Temperature 22.5°C. 11/22/32.

steeply than prior to the calcium treatment, however, so that 80 to 100 per cent recovery of irritability occurs at a definitely shorter interval than in the normal nerve. The effects on refractoriness may be reversed by returning the nerve to Ringer's solution; a new determination now shows a longer refractory period than in the fresh nerve and thus emphasizes the intervening shortening with calcium (fig. 4 E).

If both stimuli are applied at the same point of a calcium-treated nerve, the absolutely refractory period seems to be markedly prolonged, sometimes being twice as long as in the normal nerve, while the relatively refractory period seems not to be shortened significantly (fig. 4 F). This greater delay in recovery of irritability must be due to a local effect, since it occurs only at the stimulated point, and it obviously has nothing to do with true refractoriness. Its counterpart is not seen in potassium-treated nerve, in which the experimental results are the same wherever the stimuli are applied. The difference in the prolongation of the absolutely refractory period by potassium in figure 4 A and C is due to the stronger solution used in the experiment plotted in the latter, not to the difference in the method of stimulation.

The supernormal phase. The supernormal phase was recorded with an induction shock as the testing stimulus, usually by measuring the change in primary circuit resistance necessary to maintain a certain amplitude of the second response (threshold or low submaximal (8)). A few determinations were also made by measuring the change in height of response to a given submaximal testing shock, the conditioning shock being maximal. In all determinations, the conditioning and testing shocks were applied at different pairs of electrodes, 11 to 12 mm. apart.

The supernormal phase following the refractory period is known to develop with the appearance of after-potential. The potassium ion, because of its decrease of the after-potential and its lengthening of the relatively refractory period, would be expected to reduce the supernormal phase, while the calcium ion, because of its opposite effects on the after-potential and the relatively refractory period, would be expected to increase this phase. As a matter of fact, supernormality disappears altogether under potassium (fig. 4 B), while with calcium it begins earlier and lasts longer, but does not seem to be of greater degree than is attained in normal nerve at pH = 7.6 on standing for a short time, or by such experimentation as is involved in the determination of the refractory period (fig. 4 G, H). The earlier beginning is directly connected with the shortening of the refractory period, while the later ending corresponds with the prolongation of the after-potential. The apparent absence of a greater degree of supernormality in the calcium-treated nerve indicates that while refractoriness and after-potential may together control the duration of the supernormal phase, its magnitude is determined by a third and un-

known factor; the existence of such a factor has been suggested also by the cases of normal or veratrinized nerves showing considerable or large after-potentials, but little or no supernormality.

The length of the interval between the shock and the maximum of the supernormality is of some interest. As measured by the method of changing the resistance in the primary circuit, the supernormality does not rise to a sharp maximum, but has a very much rounded form, the exact top of which is difficult to locate. It is probable however that the maximum supernormality in the normal nerve falls somewhat later than the crest of the after-potential, and it has seemed that this difference might be ascribed to the influence of refractoriness. In view of the shortening of the refractory period by calcium, coincidence of maximum supernormality and after-potential crest might occur in the calcium-treated nerve, particularly as the after-potential crest is always somewhat delayed by calcium. Experiments show that the maximum supernormality occurs at a somewhat shorter interval after the stimulus in the calcium-treated than in the normal nerve; in fact it falls at about the time when the after-potential reaches its crest in the normal response, but as the calcium has delayed this crest there is no coincidence. Even when the after-potential crest has been very greatly delayed, as in the experiment represented in figure 4 G when it had moved out to 24σ after the shock before many of the measurements of the supernormal phase were made, the maximum degree of supernormality is still found very early (3–6σ in the winter frog nerves used in these experiments). The position of the maximum supernormality is therefore determined rather by the end of the relatively refractory period than by the after-potential crest; and a more gradual decline from this earlier and no-higher-than-normal maximum, takes care of the increased duration demanded by the increased duration of the after-potential.

Conduction rate. No detailed study was made of the effect of the cations on the velocity of conduction, as Rosenberg and Kitayama have recently shown that excess of either potassium or calcium decreases it. The experiments here reported confirm this finding in general, though in contrast to the results of these workers they show greater slowing with calcium than with potassium. Solutions in which the concentration of either the potassium or the calcium ion was raised to ten times its concentration in Ringer's solution usually depressed the conduction rate 15 to 25 per cent in the course of half an hour; greater slowing than this was not observed with potassium treatment, but more intense calcium treatment frequently lowered the conduction rate 40 or even 50 per cent. The lowered velocity of conduction was accompanied by lowered spike height in the case of potassium excess, and by lowered irritability and sometimes lowered spike height as well, in the case of calcium excess; and an adequate theoretical explanation is therefore available for both cases.

EFFECTS OF OTHER IONS. The trivalent aluminium ion was the only other cation considered, because of the impossibility of preparing others in sufficient concentrations at approximate neutrality. The hydrogen ion concentration necessary to maintain aluminium chloride in isotonic solution (pH = 4.1) greatly increases the after-potential, and the presence of the Al^{+++} ion made no difference in this phenomenon. Therefore although the irritability and the height of the spike-potential were decreased by the aluminium, this ion was not investigated further.

The great sensitivity of the after-potential to univalent and bivalent cations makes its indifference to its anionic environment interesting if not unexpected in view of the similar condition in regard to other better known nerve functions (25), (19). Substitution of sodium iodide for all the sodium chloride in ordinary Ringer's solution produces no significant change of after-potential; and even when all the chloride ion in Ringer's is replaced by iodine, no significant change occurs for at least an hour. The decrease of after-potential which may occur after two hours or more is probably not a direct action of the iodine ion. The thiocyanate ion (applied in 1 per cent solution or in Ringer's solution with the sodium chloride replaced by sodium thiocyanate) is equally inactive, and so is the sulphate ion, which differs from the other two not only in its valence, but also in its position in the Hofmeister series. The only two anions found to have any action were the fluoride and oxalate ions, and these produced potassium-like after-potentials and potassium-like depressions of the spike-potentials (illustrated for sodium fluoride in fig. 5). These anions are powerful calcium precipitants, and their effects on the nerve are promptly reversed by supplying calcium; their ability to precipitate the calcium of the nerve is ample explanation of their activity.

Fig. 5. Effect of sodium fluoride on after-potential. Records superimposed for printing (× ⅔). Ordinates 1 mm. = 7.5 $\mu\sigma$; abscissae, time in 25σ intervals. *1* normal; *2*, 50 minutes after isotonic sodium fluoride applied; height of spike reduced to 39 per cent of normal. Subsequent treatment with isotonic calcium chloride restored the after-potential to normal form and increased the spike height to 45 per cent normal. The small waves in the line are fortuitous. 5/2/32.

DISCUSSION. The list of electrical properties of nerve previously reported to change in one direction with excess of univalent cations and in the other with excess of bivalent cations, includes the size of the electrical response (Waller), the irritability to induction shocks, to slowly rising currents and to constant currents of varying durations (Jahn, Blumenfeldt, Höber and Strohe, Misske) and the restoration, under the cathode or the

anode of a constant current, of conduction destroyed by cationic imbalance (Woronzow). The experiments reported in this paper show that the size and duration of the after-potential and the duration of the relatively refractory period are similarly sensitive to changes in the cationic environment.

The change in the after-potential is undoubtedly the chief factor in the "augmentation of response" observed by Waller after applying salts of bivalent cations to nerve; the "deterioration of response" he reports with univalent cations is of course due to the depressing effect of these ions on both the after-potential and the spike. The increase in the after-potential with excess of bivalent cations is very evident in Boruttau's string galvanometer records of nerve response, though he himself places more emphasis on the changes induced in the rapid part of the action potential; these changes may be ascribed to temporal dispersion, in view of the present evidence against any change in the time relations of the spike due to variations of the cation ratio.

Wassiliew and Mogendowitsch, in following up the work of Wedenski on the response of muscle stimulated through its nerve by a series of subthreshold tetanic shocks plus a single super-threshold induction shock, have found that the peculiar form of muscle response seen in a normal preparation may be transformed into a typical twitch by increasing the potassium in the fluid bathing the nerve, or into a tetanic response by increasing the calcium. At the rates of stimulation used by these workers (50–100 interruptions per second), the successive shocks would fall in the critical interval during which refractoriness or subthreshold cathodal depression (see below) in the nerve under potassium treatment would become maximal or nearly maximal supernormality with calcium, and the difference in the muscle response is thus adequately explained by changes known to be induced in the nerve.

The change in the relatively refractory period does not seem at first sight to be directly related to any of the other cation-sensitive functions. However, Erlanger and Blair (8) have recently suggested that the relatively refractory period, like the phase of decreased irritability after a subthreshold break induction shock, is an expression of postcathodal depression. If this conclusion be adopted, then the shortening of the relatively refractory period by calcium is very obviously related to the fact that conduction of the nerve impulse prevented by excess of this ion is restored under the cathode of a polarizing current (30); and the lengthening of the relatively refractory period by potassium and the restoration under the anode of conduction blocked by this ion are similarly related actions. The shortening of the relatively refractory period in nerve by excess of calcium recalls not only Bazett's similar finding in skeletal muscle, but also Lucas' observation (23) of the shortening of the summation interval by this ion;

the lengthening of the summation interval by cathodal polarization, reported by Erlanger and Blair (8), is the obvious complement to the latter phenomenon. The similarity of excess of potassium to cathodal polarization and of excess of calcium to anodal polarization is further evident in their effects on the irritability of nerve; the early increase of irritability with univalent cations is undoubtedly to be regarded as their characteristic action and agrees with the well-known cathodal enhancement of irritability.

The changes produced by excess of potassium or of calcium in the nerve functions here considered are not however identical in all respects with those reported for cathodal and anodal polarization (4), (9); 1, no increase of conduction rate has been observed with potassium; 2, no shortening of the absolutely refractory period has been observed with calcium; 3, nor has this ion been found to increase the amplitude of the action-potential. Further, 4, the time-relations of the spike remain unchanged with excess of either cation. Of these differences, 1, the cathodal increase of conduction rate occurs only when the impulse approaches the cathode through the anodal area where it undergoes the anodal increase in amplitude; since an impulse coming from normal nerve is not accelerated as it approaches the cathode, it is not surprising that an impulse depressed by traversing a potassium-treated nerve shows a subnormal conduction rate. As is well known, the conduction rate varies with the two factors, height of the action-potential and irritability of the tissue; it is evident that potassium does not increase the latter sufficiently to overcome its depression of the former. 2, and 3. The anodal decrease of the absolutely refractory period is slight, and the anodal increase of amplitude of potential though more marked is nevertheless seen only when the polarizing current is weak. The explanation of the differences in the action of calcium and anodal polarization on these functions is not obvious; perhaps these differences will be found to disappear with further experimentation. 4. The changes observed in the time-relations of the spike potential with polarization are a slight prolongation of the rising-phase with either cathodal or anodal polarization, and a marked prolongation of the falling-phase with anodal polarization and a considerable shortening of this phase with cathodal polarization. Erlanger and Blair (9, p. 145) interpret the oberved prolongation of the rising-phase with cathodal polarization as due to the wider range of latencies in this condition, and make the assumption that the axon spike potential is actually shortened in both its rising and falling phases. If the observed lengthening of both phases by anodal polarization is also considered an expression of a change in the axon spike potential, then the time-relations of the spike are oppositely affected by cathodal and anodal polarization, in direct contrast to their constancy under excess of univalent or bivalent cations. It seems possible, however, in view of the other points of simi-

larity between the effects of polarization and cations, that the after-potential may be lengthened by anodal polarization as by calcium, and shortened by cathodal polarization as by potassium; if so, the effects on the falling-phase of the spike-potential observed in polarized nerve may prove to be due at least in part to after-potential changes, and the time-relations of the spike may after all not be a touchstone differentiating the effects of polarization from those of variations in the cation ratio.

Further experimental studies are needed before the limits of the similarity between cathodal polarization and potassium excess, and between anodal polarization and calcium excess can be definitely stated. These agents certainly do not exert identical actions in every case. The demarcation potential for instance is a function oppositely affected by cathodal and anodal polarization, but changed in the same direction by excess of either univalent or bivalent cations; the effect of potassium is much more marked, but that of calcium is certainly in the same direction (25), (19), (3). In connection with the demarcation potential, attention may be called again to the fact brought out in the introduction, that this potential and the after-potential certainly bear no simple inverse relationship to each other; while it is true that veratrine and calcium both increase the after-potential and decrease the demarcation potential somewhat, potassium, which decreases the latter much more, decreases the former as well.

The ability to produce electrotonic currents is another function of nerve which might be expected to vary in opposite directions with excess of univalent or bivalent cations, but which actually varies always in one direction: Höber and Strohe report increases in both anelectrotonic and catelectrotonic currents under the influence of either group of cations.

The fundamental change underlying the similar actions of polarization and cations can not be determined at present, any more than can the limits of the similarity of these two agents. According to the generally accepted membrane theory of nerve action, and the rôle traditionally assigned to ionic factors, the underlying change would be considered a change in the permeability or the state of polarization of the plasma membrane. But aside from the known cases in which the effects of polarization and cations are different, there are other difficulties in the way of this explanation in its simplest form. For one thing, the constancy of the time-relations of the spike when the cation ratio is changed necessitates subsidiary hypotheses or redefinition of the "plasma membrane." Further, if the after-potential be, as is probable, one of the functions affected by cathodal polarization as by potassium, and by anodal polarization as by calcium, then some of these "membrane-functions" must be controlled also by other factors, for veratrine is known to act like potassium on the irritability and the relatively refractory period, but like calcium on the after-potential and the absolutely refractory period.

In the case of the after-potential there are other reasons for thinking that it is not controlled solely by the condition of a membrane, but by other factors as well. Certain of its properties, particularly its sensitivity to asphyxia and the positive temperature coefficient of its rising-phase, point to a chemical basis for this potential; and it has been regarded as associated with the production and disappearance of secondary (oxidized) catabolites (13). The production of catabolites still seems the best hypothesis to account for the temperature coefficient and the long duration of the rising-phase; and the reason that oxygen is necessary may therefore still be that it is essential for the production of the active catabolites. On the other hand, the other possibility—that the necessity for oxygen is due to its function in maintaining a certain state of the nerve, presumably a definite membrane condition suitable for the appearance of after-potential—is certainly not weakened by the above evidence of the importance of a membrane.

Whatever may be the rôle of oxygen in the production of after-potential, the fact that this potential is also directly controlled by the cationic environment offers certain suggestions regarding it. 1. In the first place, the increase of after-potential with stimulation may now be interpreted as due to a shift in the cation ratio at some critical structure in the nerve—in other words, the secondary catabolites that have to be produced whether by oxidation or otherwise, before the after-potential is manifested, may be nothing more than calcium ions strategically placed. The ramifications of this suggestion in connection with the various theories of stimulation by a shift in the cation ratio, will not be considered here. 2. In the second place, the tendency of the after-potential of isolated frog nerve to increase with time may be connected with an excess of calcium in the Ringer's solution used as "normal." The amount of calcium may be excessive in relation to the after-potential only; other nerve functions less sensitive to the cation-ratio may show no change in the same solution. If after-potential be the electrical sign of a process or a condition essential to the recovery from stimulation of isolated frog nerve, it is evident that the cation ratio must be such as to produce or permit the development of after-potential; otherwise incomplete recovery will lead to depression of nerve response. In view of the close connection between after-potential and irritability it is interesting that Lucas (21) observed in amphibian muscle in Ringer's solution, slow changes in certain irritability factors (liminal current gradient and liminal current duration) which he showed to be dependent upon the amount of calcium in the solution. 3. It is a striking fact that agents known to reduce the after-potential—potassium, narcosis (15), asphyxia (1)—all depress the spike very greatly, while the after-potential-increasing agents—veratrine, bivalent cations—are relatively non-toxic to the nerve response. Certain experimental observations—e.g., the restoration by

calcium of the height of the potassium-depressed spike before the after-potential is restored—prevent attributing the spike-depression to the inhibition of the recovery-process associated with the after-potential. The parallelism between the effects of any agent on the two potentials may be better interpreted as due to a similar action in the two cases, and the similarity is more likely to be found in modification of a membrane, whether or not the membrane involved is the same in the two cases, than in interference in the presumably widely different chemistry of the two processes. 4. The seasonal difference in after-potential, its much greater development in nerves from winter than from summer frogs, may also be connected with its sensitivity to cations, if, as has been reported (7, p. 357; 18, footnote p. 113) the calcium content of serum is higher in summer than in winter frogs. The nerves coming from and adapted to the calcium-rich environment might well fail to develop after-potential in Ringer's solution containing enough calcium to produce or permit the development of considerable after-potential by a nerve adapted to a calcium-poor environment.

The arrangement of the univalent cations in the order of their activity on the after-potential is substantially that observed with many nerve functions, although the division into two groups is unusually well marked in the case of the after-potential. The same division is found in at least one non-nervous case: Gellhorn reports that in depressing the motility of the spermatozoa of Rana temporaria, the rubidium, potassium and ammonium ions are about equally active, and many times stronger than the sodium, caesium and lithium ions, which again are about equal in activity.

SUMMARY

Increase of the potassium ion content to 3–10 times its concentration in Ringer's solution decreases the magnitude of the after-potential of isolated frog nerve; the decrease is relatively greater than that of the spike height. The duration of the after-potential is reduced to 20–50σ. Increase of the calcium ion content to 9–13 times its concentration in Ringer's solution increases the magnitude of the after-potential up to as much as 4 per cent of the spike-potential with no effect on, or a slight decrease of, the spike height. The rising-time of the after-potential is prolonged to 10–30σ, and the total duration is 200 to 300σ.

The activity of the rubidium and ammonium ions resembles that of the potassium ion. The sodium, caesium and lithium ions are very much less active. The activity of the barium, magnesium and strontium ions resembles that of the calcium ion, though barium is somewhat more active. The beryllium and aluminium ions were not found to have any effect on the after-potential.

Anions (except calcium precipitants) have no effect on the after-potential.

The time-relations of the spike are not affected by excess of either univalent or bivalent cations.

Accompanying the known increase of irritability with excess of univalent cations, and its decrease with excess of bivalent cations, there occurs a prolongation of the absolutely and relatively refractory periods with univalent cations, and a shortening of the relatively refractory period and apparently no change of the absolutely refractory period with bivalent cations.

Excess of the potassium ion removes the supernormal phase; excess of the calcium ion causes this phase to begin earlier and last later than in normal nerve.

There is an extensive similarity between the changes produced in various nerve functions by cathodal polarization and by excess of univalent cations on the one hand, and by anodal polarization and by excess of bivalent cations on the other.

The sensitivity of the after-potential to the cationic environment indicates that it is a membrane function. The experimental fact and this conclusion from it are discussed in relation to their bearing on the nature of after-potential.

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OSMOTIC RELATIONS BETWEEN BLOOD AND BODY FLUIDS

III. The Osmotic Relation of Blood and Aqueous Humor

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Recent investigations upon the physical chemistry of the aqueous humor, notably those of Duke-Elder (1927), have presented convincing evidence that the mechanism of formation of this fluid is that of dialysis through the capillary membranes of the eye. Chemical measurements of the concentration of the various constituents of the aqueous humor as compared with the blood support this view (Cohen, Killian and Metzger, 1926; Duke-Elder, 1927, and Yudkin, 1928). The assumption that the aqueous humor is formed by a passage of various constituents of the blood through the capillary walls necessitates that these fluids be in osmotic equilibrium. Consequently accurate osmotic pressure measurements of these two fluids are essential for the proof of this physical-chemical origin of the aqueous humor.

Previous investigations on the osmotic pressure of the aqueous humor have been handicapped by the fact that it was necessary to use pooled samples of fluid from several eyes in order to obtain an accurate measurement. The osmotic pressure of the aqueous humor obtained by such a procedure was found to be approximately the same as that of the blood. The limitation of the accuracy of such a measurement lies in the fact that there may be rather wide differences in the state of hydration and the osmotic pressures of the body fluids of the several animals employed. Duke-Elder (1927) avoided this error by using the aqueous humor of a single subject. However, handicapped by a small amount of fluid, he was unable to measure total osmotic pressure. Instead he determined the difference in colloidal osmotic pressure between the blood and aqueous humor of a single animal. He then estimated any difference that might exist in osmotic pressure due to the more active non-colloidal elements of these fluids by measuring changes in their conductivity when separated by a semi-permeable membrane. Although accuracy of such a procedure

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cannot be questioned, the advantage of direct measurements of osmotic pressure are obvious. Therefore, it was thought of interest to extend these observations, using a new and accurate method, with which one can measure the osmotic pressure of the aqueous humor of a single eye, and including a sufficient number of cases to allow statistical treatment of the data.

EXPERIMENTAL PROCEDURE. Dogs were used as the experimental subjects. The cornea of the eye was anesthetized with cocaine and the fluid of the anterior chamber withdrawn. Simultaneously, a blood sample was taken from the femoral artery. The blood was defibrinated by whipping with glass beads. Osmotic pressure determinations were made upon this defibrinated whole blood. The vapor pressure method of A. V. Hill (1930), described in detail by Margaria (1930), was employed.

RESULTS. A comparison was made of the osmotic pressure of the blood and aqueous humor of 27 normal dogs. The average osmotic pressure of all the blood samples examined was equivalent to that of a 0.926 per cent NaCl solution, as compared with the average figure of 0.924 per cent for the eye fluids. However greater variations are noted in the deviations obtained between the values for the blood and aqueous humor of a single animal. The average deviation between the osmotic pressure of the blood and aqueous humor in this series of normal animals was equivalent to a difference represented by the osmotic pressure of a 0.012 per cent NaCl solution, whereas the standard deviation was 0.018 per cent. Such agreement between the osmotic pressure of the circulating fluid and that of the aqueous humor strongly indicates that the formation of the latter fluid is by means of a physical-chemical exchange from the blood stream.

The existence of such an equilibrium implies the fact that any change produced in the osmotic pressure of the blood should be accompanied by corresponding changes in the osmotic pressure of the aqueous humor. A study of the dynamics of such an equilibration might be of value for a more complete understanding of the variations of intraocular pressure observed in the anterior chamber. Such pressure changes following the intravenous injection of hypertonic solutions have been recorded by Duke-Elder (1926). However, no measurements have been made upon the respective osmotic pressures of aqueous humor and blood when the osmotic pressure of the latter fluid has been caused to change appreciably.

Osmotic changes in the aqueous humor following the intravenous administration of hyper- and hypotonic solutions. Two series of experiments were performed in which measurements were made upon the osmotic pressure of the aqueous humor following the administration of either hypertonic saline or water. In one series of experiments dogs under amytal anesthesia received intravenously, following the withdrawal of a normal blood sample, 5 cc. per kilo of 10 per cent saline. After allowing 30 minutes for equilibration, a sample of blood from the femoral artery was taken simul-

taneously with the withdrawal of fluid from the anterior chamber of the eye. No sample of ocular fluid was obtained before the administration of saline inasmuch as the subsequent procedure required the withdrawal of two samples of aqueous humor from intact eyes. It is a known fact that the removal of fluid from the anterior chamber of the eye produces a pathological condition resulting in the reformation of fluid of a changed chemical composition (Yudkin, 1926). Therefore, the osmotic pressure of the aqueous humor before injection of hypertonic saline was considered to be approximately the same as the osmotic pressure of the normal blood sample. Results obtained from normal animals justify such an assumption. The osmotic changes of the blood and aqueous humor following the salt injection are summarized in table 1.

As can readily be seen from the table, abrupt increases in the osmotic pressure of the blood are immediately reflected in the osmotic pressure of

TABLE 1

EXPERIMENT	OSMOTIC PRESSURE NORMAL BLOOD	BLOOD OSMOTIC PRESSURE AFTER NaCl	AQUEOUS OSMOTIC PRESSURE AFTER NaCl	BLOOD OSMOTIC PRESSURE AFTER H_2O	AQUEOUS H_2O
1	0.910	0.985	0.954	0.847	0.908
2	0.907	1.020	0.998	0.829	0.934
3	0.931	1.024	1.030	0.851	0.897
4	0.914	0.945	0.976	0.905	0.904
5	0.898	0.968	0.944	0.855	0.895

Osmotic pressure is expressed as per cent concentration of an osmotically equivalent NaCl solution.

the aqueous humor. In these experiments, 30 minutes after the administration of hypertonic saline, physical-chemical equilibrium had once more caused these fluids to be approximately isotonic, although in four of the experiments, due to the limited time for equilibration, the osmotic pressure of the aqueous humor was slightly lower than that of the blood.

Evidence that the eye fluids may increase in osmotic pressure more readily than decrease is indicated by the following series of observations. After measurements were made upon the above animals dehydrated by means of hypertonic saline, opposite changes in the blood osmotic pressure were produced by the intravenous administration of distilled water (50 cc. kilo). The water was given over a period of 90 minutes to prevent too extensive hemolysis. Equilibration was then allowed to proceed for an additional 30 minutes. Osmotic changes and equilibration therefore were occurring over a period of 120 minutes, as contrasted to 30 minutes in the above experiment. The results of this experiment are also summarized in table 1. Here again is observed the obvious tendency for the osmotic

pressure of the aqueous humor to change parallel with that of the blood. However, as contrasted with the results obtained after the administration of salt, a reduction in the osmotic pressure of the fluid in the anterior chamber of the eye is accomplished more slowly. In spite of the longer equilibration time as contrasted with the above experiment, the osmotic pressure of the blood and aqueous humor are not as closely approximated, the blood being definitely hypotonic.

This observation is further substantiated by another series of experiments made upon dogs without anesthesia. Fluid, either hypertonic saline or distilled water, was administered by stomach tube, after having obtained normal blood and aqueous humor samples. Ninety minutes after the administration of either fluid, samples of blood and aqueous humor were again taken. The results of this experiment appear in table 2. Only two observations were made following the saline administration. In

TABLE 2

OSMOTIC PRESSURE NORMAL BLOOD	BLOOD OSMOTIC PRESSURE AFTER NaCl	AQUEOUS OSMOTIC PRESSURE AFTER NaCl	BLOOD OSMOTIC PRESSURE AFTER H_2O	AQUEOUS H_2O
0.928	1.018	1.023		
0.909	0.955	0.945		
0.901			0.834	0.873
0.929			0.848	0.892
0.908			0.839	0.860
0.936			0.887	0.890
0.887			0.811	0.845

Osmotic pressure is expressed as per cent concentration of an osmotically equivalent NaCl solution.

each case, however, there was a marked rise in the osmotic pressure of the blood accompanied by a similar increase in the osmotic pressure of the aqueous humor, with the result that both fluids remained practically isotonic. Following the intake of water there is observed a decrease in both the osmotic pressures of the blood and aqueous humor. However, in the majority of these water experiments the dilution observed in the blood was much greater than that of the aqueous humor, the end result being an aqueous humor definitely hypertonic to the circulating fluid.

Discussion. The fact that the aqueous humor is formed by a passage of constituents of the blood stream through this capillary membrane is granted by most investigators (Mestrezat and Magitot, 1922; van Creveld, 1921; Asher, 1922; Gala, 1924; Wittenstein and Gaedertz, 1927; Yudkin, 1928; Duke-Elder, 1927, and Tron, 1926) although a few still support the hypothesis that the aqueous humor may be of glandular origin (Seidel, 1927). However, the above experiments, which clearly demonstrate an

active osmotic equilibration between the fluid of the anterior chamber of the eye and the blood stream, are in favor of the former and more widely accepted theory. Opinions concerning the circulation of the aqueous humor and the manner in which it might possibly escape from the anterior chamber are more contradictory. One group of workers explains the entire circulation of this fluid by means of Starling's hypothesis, (Henderson and Starling, 1908) namely, that the aqueous humor of the eye can only return to the blood stream by a passage back through the capillary wall at a point where the attracting osmotic pressure of the proteins within the vascular system is greater than the hydrostatic pressure which was the original force concerned with their formation. Other workers (Leber, 1903) postulate the removal of fluid from the anterior chamber by way of the canal of Schlemm, this means of exit being especially effective when the pressure in the anterior chamber is above normal. It was noticed in this investigation that the aqueous humor equilibrated with the blood stream with facility when the latter was made hypertonic as contrasted with the slower and more anisotonic approximation when water was used. Following the administration of salt, the osmotic pressure of the eye can adjust itself to that of the blood either by the passage of salt into the anterior chamber, or by the migration of water into the circulating fluid. This will be associated with a reduction in intraocular pressure as has been pointed out by Duke-Elder (1926). On the other hand, after water administration the passage of the above constituents will be in the opposite direction, water entering the anterior chamber and raising its pressure. This increased pressure will, at some level, resist any further entrance of fluid, causing complete osmotic equilibration to be dependent upon salt passing into the blood. This is believed to be the reason for the discrepancies in osmotic pressure following dilution of the blood stream observed in these experiments. It also would contradict the conception of the functional capacity of the canal of Schlemm, for if fluid was removed from the eye by this means it is reasonable to suppose that equilibration would proceed with greater facility if relatively unimpeded by the accumulation of fluid in the anterior chamber. It has been pointed out by clinicians, however, that the canal of Schlemm may be occluded by pressure from the vitreous body, which they believe becomes turgescent in conditions of abnormally increased intraocular pressure, but such a view has not been definitely corroborated by experimentation.

The passage of water from the anterior chamber of the eye into the blood stream with the resultant decrease in intraocular tension following the administration of hypertonic solutions suggests this procedure as a clinical measure for the relief of increased ocular tension. In view of the observations reported in this paper, however, such a measure would be of doubtful value. The experiments described above indicate that within 30 minutes

after the intravenous administration of hypertonic NaCl the osmotic pressure of the aqueous humor has approximated that of the blood. Once this equilibration has taken place, despite the high osmotic pressure of the blood, there are no forces acting to prevent the return of the intraocular tension to its former level. Furthermore, the increased blood volume with its possible accompanying increased capillary hydrostatic pressure in addition to a lower concentration of serum protein may be a factor in raising the ocular tension above its original level. If the elimination of salt from the body by the kidney exceeded the rate at which it could diffuse from the fluid in the anterior chamber back into the blood stream, this would constitute still another factor operating to increase rather than decrease intraocular tension. Duke-Elder (1926), Lambert and Wolf (1929), Hertel (1915) and others have practised the intravenous injection of hypertonic saline to relieve intraocular tension. Aside from its original and transient effect, the procedure would seem to be contraindicated.

SUMMARY AND CONCLUSIONS

Measurements of the osmotic pressure of the aqueous humor and arterial blood obtained simultaneously from normal dogs revealed the two fluids to be isotonic within the experimental error of the method of determination (± 0.5 per cent). The intravenous and oral administration of hypertonic saline or water resulted in an increase and decrease in the osmotic pressure of the blood accompanied by the concomitant changes in the osmotic pressure of the aqueous humor. Observations indicated that osmotic equilibration between these two fluids proceeded more rapidly and with greater facility when the osmotic pressure of the blood was increased than when the opposite change was effected. This discrepancy is believed to be due to the resistance to the passage of fluid into the anterior chamber because of the increased intraocular pressure caused by its entrance.

We wish to acknowledge our indebtedness to Dr. George R. Cowgill for his interest in this problem.

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STUDIES ON THE PHYSIOLOGY OF THE LIVER

XXV. Allantoin and Uric Acid Following Total Removal of the Liver

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In a previous study on the physiology of the liver, we reported that destruction of uric acid was entirely dependent on the presence of the liver in the dog. We found that large amounts of uric acid appeared in the urine of dogs following hepatectomy, and that there was also an increase in the uric acid content of the blood. Uric acid injected intravenously into such animals was almost completely recovered in the urine, in contrast to the small amount recovered under similar conditions in the normal animal. Determinations of the uric acid content of the blood and tissues of dehepatized, nephrectomized animals several hours after injection of uric acid revealed that the entire amount was retained in the body, and that little of it could be destroyed. The excretion of uric acid by the dehepatized animal appeared to be of the same magnitude as the allantoin excreted by the normal animal.

At the time of the foregoing experiments we were unable to obtain satisfactory results from determinations of allantoin in the urine of dehepatized dogs. Recently, we have used the method of Larson for these determinations. These studies confirm our previous work on the relation of the liver to uric acid metabolism, and add the additional evidence of the apparent cessation of formation of allantoin in the absence of the liver.

Methods. The liver was removed in three stages, as previously described. After operation the animals were maintained in normal appearing condition by the intravenous administration of glucose. Control normal animals were anesthetized in the same way, allowed to recover, and received the same amount of glucose by intravenous injection. Specimens of blood were obtained from the jugular vein. Three-hour specimens of urine were obtained by catheterization at the beginning and end of each period.

Determinations of uric acid were made on the blood and urine by the method of Folin. Allantoin in the urine was determined by the method of Larson. In our preliminary experiments with this method, almost 100

per cent of allantoin added to dog urine was recovered, but losses of allantoin occurred when pure solutions of allantoin were used. We found that the presence of sugar in the urine increased the reaction for allantoin. Preliminary treatment of the urine with yeast was found to be satisfactory for removal of glucose, and did not interfere with the recovery of added

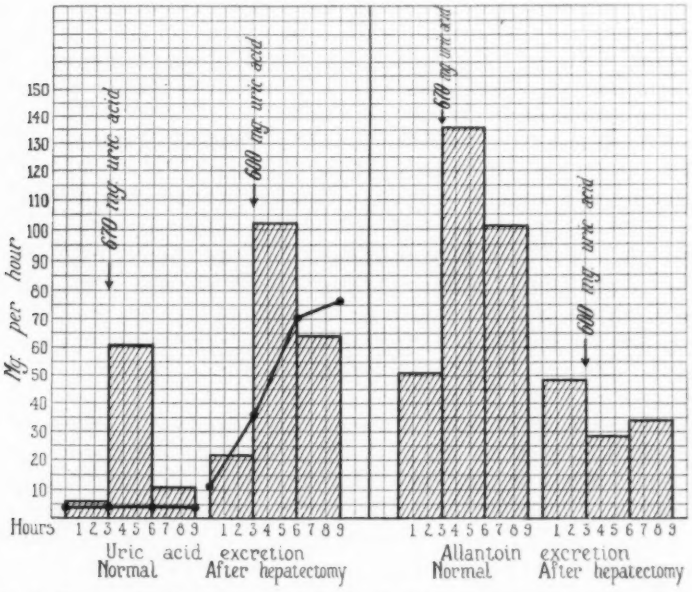

Fig. 1. Curves showing the excretion of uric acid and allantoin following intravenous injection of 50 mgm. of uric acid for each kilogram body weight in the normal and in the dehepatized dog. The increased excretion of uric acid and the diminished excretion of allantoin after hepatectomy may be noted. The solid line represents the uric acid content of the blood expressed as milligrams of uric acid for each liter of blood. The normal animal excreted 27 per cent of the injected uric acid unchanged, and 64 per cent was excreted as allantoin. Following removal of the liver the same animal excreted 61 per cent of the uric acid unchanged, and showed a decrease instead of an increase in the excretion of allantoin. The volume of urine excreted was similar in both experiments.

allantoin. It appears to us that the values we obtained represent all the added allantoin, but that the urine of dogs contains substances which give values slightly higher than the true allantoin content.

Results. The studies of uric acid in this series were similar to those previously reported. The normal dog has but traces of uric acid in the blood, and excretes less than 100 mgm. in the urine in twenty-four hours.

Fifty milligrams of uric acid for each kilogram of body weight, injected intravenously, completely disappeared from the blood within one hour, and increased the excretion of uric acid for about six hours, so that from 5 to 30 per cent of the uric acid injected was recovered unchanged in the urine. The excretion of allantoin is increased by the administration of uric acid. The greater part of the allantoin from uric acid is excreted within three hours, and within nine hours after injection the increased allantoin excreted is equivalent to 50 to 90 per cent of the uric acid administered. Similar results were obtained with normal animals that had been etherized, and that subsequently had been given glucose in amounts similar to that given the dehepatized animals.

In the dehepatized animal maintained by injections of glucose, there is a much greater rise than normal in the uric acid content of the blood and in the uric acid content of the urine. If the flow of urine is well maintained, the amount of uric acid excreted each hour is fairly constant. Injection of 50 mgm. of uric acid for each kilogram of body weight gives a greater rise in the excretion of uric acid, so that 50 to 90 per cent of the uric acid injected appears to be excreted in the urine within twelve hours afterward. If consideration is given to the increased uric acid content of the blood and tissues at this time, almost quantitative recovery of the uric acid may be calculated.

The excretion of allantoin of the liverless dog is not increased by the intravenous injection of uric acid, but is definitely decreased following complete removal of the liver. This decrease in excretion of allantoin appears to be quite similar to the decrease in excretion of urea. The excretion of urea after total removal of the liver is accounted for by the decreased urea content of the blood and tissues, and it is obvious that no urea is formed after the liver is removed. The constantly decreasing excretion of allantoin after removal of the liver may be considered as evidence of the cessation of formation of allantoin in the absence of the liver.

After total removal of the liver the formation of uric acid progresses at a rate comparable to that indicated by the excretion of allantoin by the normal dog. The destruction of uric acid and the formation of allantoin is entirely prevented by the removal of the liver.

REPRESENTATIVE PROTOCOLS. *Dog. 1.* An adult male terrier weighed 11.0 kgm. after a fast of eighteen hours. The animal was catheterized and given an intravenous injection of 100 cc. of 10 per cent glucose. The injections of glucose were repeated every ninety minutes, and the urine was drawn by catheterization at intervals of three hours. At the end of the first three-hour period 550 mgm. of uric acid (50 mgm. for each kgm. of body weight) were injected at the same time as the glucose for that period. The excretion for each three-hour period was as follows:

PERIOD	URINE VOLUME	URIC ACID	ALLANTOIN
	cc.	*mgm.*	*mgm*
1	100	16	86
2	150	19	269
3	200	17	233
4	160	14	215

In the nine hours following injection of uric acid, 717 mgm. of allantoin were excreted. Assuming a basal excretion of 86 mgm. for each three-hour period, 459 mgm. of allantoin were excreted in excess of the basal secretion. Four hundred fifty-nine milligrams of allantoin are equivalent to 488 mgm. of uric acid, and represent a recovery of almost 90 per cent of the injected 550 mgm. of uric acid as allantoin in the urine.

Dog 2. An adult female hound weighed 20.0 kgm. after a fast of eighteen hours. The animal was catheterized, and each hour for fifteen hours was given continuous intravenous injection of 80 cc. of 5 per cent glucose. The urine was collected at three-hour periods. At the end of the first three-hour period, 500 mgm. of uric acid each hour were injected for two hours. The excretion for each three-hour period was as follows:

PERIOD	URINE VOLUME	URIC ACID	ALLANTOIN
	cc.	*mgm.*	*mgm.*
1	190	12	131
2	270	93	590
3	130	66	231
4	385	22	189
5	160	13	121

In the nine hours following injection of uric acid, 1,010 mgm. of allantoin were excreted. Assuming a basal excretion of 131 mgm. for each three-hour period, 617 mgm. of allantoin were excreted in excess of the basal secretion. Six hundred seventeen milligrams of allantoin are equivalent to 657 mgm. of uric acid, which represents a recovery of 65.7 per cent of the 1,000 mgm. of uric acid injected, as allantoin in the urine. One hundred forty-five milligrams of uric acid (14.5 per cent) were recovered unchanged from the urine.

Dog 3. An adult male bulldog weighed 29.8 kgm. one hour after hepatectomy. Each hour for fourteen hours the animal was given a continuous intravenous injection of 0.1 gram of glucose for each kilogram body weight. The urine was collected every three hours. The excretion of uric acid and allantoin was as follows.

PERIOD	URINE VOLUME	URIC ACID	ALLANTOIN
	cc.	*mgm.*	*mgm.*
1	100	148	74
2	100	194	53
3	100	136	44
4	100	155	48
5 (2 hrs.)	50	55	13

The large amount of uric acid excreted and the relatively small amount of allantoin excreted indicate a failure of the conversion of endogenous uric acid to allantoin in the absence of the liver.

Dog 4. A female hound weighed 17.2 kgm. one hour after hepatectomy. Each hour for twelve hours the animal was given a continuous intravenous injection of 0.2 gram of glucose for each kilogram body weight. After the first three-hour period, 300 mgm. of uric acid were injected each hour for two hours. The excretion for each three-hour period was as follows:

PERIOD	URINE VOLUME	URIC ACID	ALLANTOIN
	cc.	*mgm.*	*mgm.*
1	150	71	81
2	450	463	108
3	250	190	81
4	170	110	43

In the nine hours following injection of 600 mgm. of uric acid, 763 mgm. of uric acid were excreted. Assuming a basal excretion of 71 mgm. for each three-hour period, 550 mgm. of uric acid were excreted in excess of the basal excretion, which represents a recovery, unchanged in the urine, of 92 per cent of the 600 mgm. of uric acid injected. The excess excretion of 27 mgm. of allantoin may perhaps be accounted for by the diuresis obtained during that period, or to the cumulative error due to the large volume of urine involved. No evidence of increased excretion of allantoin was obtained in similar experiments on other dehapatized dogs.

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THE EFFECT OF FEEDING DESICCATED THYROID UPON THE SEXUAL MATURATION OF THE ALBINO RAT[1]

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In the ovaries of cretin rabbits Tatum (1) found histologically a decrease in the number of primary follicles, an increased size of the follicular space and degenerative changes in the ova. These degenerative changes of the ovary were confirmed by Kunde, Carlson and Proud (2). When 60 to 140 mgm. of desiccated thyroid were fed daily to cretin rabbits for a period of 4 to 8 weeks or until symptoms of hyperthyroidism became definitely established Kunde observed a decided increase in the number of follicles (especially primary follicles) of the ovary. A study of the effect of desiccated thyroid upon pubescence of otherwise normal rats was undertaken with the view of securing further data on the correlation of ovarian and thyroid function.

The inter-relation between thyroid and ovary has been appreciated clinically for centuries on account of the increased size of the thyroid at menstruation, deflorescence and pregnancy (3), (4). Kunde (5) and Benedict and Finn (6) used the basal metabolic rate as a criterion of thyroid function and found a slight decrease in the basal metabolic rate during menstruation in normal women. In cases with thyroid pathology, whether myxedema or hyperthyroidism, the ovarian function is disturbed so that amenorrhea or menorrhagia are reported as associated with both syndromes (7), (8), (9). Basal metabolic studies conducted on normal women during pregnancy showed a decided rise especially during the last month. Murlin (10), in studies on women and dogs, and Sandiford (11), (12) in a very careful study on one woman during two pregnancies, showed that the increased metabolic rate could be explained by the energy requirements of the fetus and accessory structures of pregnancy. Rowe (13) and Root (14) report in women the basal metabolic rate increased above the combined basals of the mother, fetus and accessories of pregnancy. Garipuy and his co-workers (15) found hyperthyroid symptoms developed in only 1 of 16 pregnant women. Therefore, they concluded that the higher basal metabolic rate during pregnancy was not due to the thyroid but due to certain products eliminated by the fetus and placenta.

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In experimental hyperthyroidism in rabbits induced by the injection of 0.02 gram of a lipoid extract of the thyroid gland daily Iscovesco (16) produced hypertrophy of the testes in the male. Hoskins (17) found only a very slight increase in weight of testes and epididymides of rats fed desiccated thyroid. Zawodowsky (18), on the other hand, reported a decrease in the weight of the testes of roosters greater than the slight decrease in body weight after feeding desiccated thyroid. He, also, examined the ovaries of hens rendered hyperthyroid and found degeneration of the ripe follicles and cessation of ovulation. Similar ovarian changes were reported by Dulzetto (19) in white rats injected with 0.4 cc. of "Endothyroidein" daily from weaning to sexual maturity.

Evans and Long (20) found no specific effects upon the oestrous cycle of the rat as a result of thyroid feeding. Lee (28) reported a slight lengthening of the oestrous cycle of rats after thyroidectomy. In the normal rat he found an average increase of 12 per cent in the basal metabolic rate during di-oestrum and pro-oestrum. Since this rise could not be produced by injecting "oestrous hormone" into ovariectomized rats the increased basal seemed to be of thyroid origin. However when Wang (21) utilized Slonaker's (22) method of determining oestrus by observing the number of revolutions run by a rat in a rotary cage he found a definite decrease in the number of revolutions during periods of thyroid feeding.

Methods. In this study 269 albino rats (204 females and 65 males) were used. Litters ranging in age from 18 to 21 days and weighing from 20 to 30 grams were divided into 2, 3 or 4 groups (depending upon the number of rats in the litter). One group of 103 females and 25 males represented the controls. These rats received only the stock diet which consisted of bread and milk daily to which meat was added every other day. Green-stuff was fed once or twice a week. A special effort was made to keep the animals in an excellent state of nutrition because Slonaker (23) and Stone (24) found that pubescence was retarded in rats on a deficient diet. The other groups were fed the same diet as the controls plus doses of desiccated thyroid varying from 0.5 to 10.0 mgm. per rat daily. The desiccated thyroid was weighed out daily and fed mixed in small pellets of bread and milk which the rats ate readily.

The criterion of pubescence in the female rat used in this study was the opening of the vagina. The immature female rat's vagina is closed by a membrane which breaks spontaneously just before the first oestrous cycle (25), (26), (27). Although Slonaker used the time when the first litter was born minus 21 days (the established period of gestation) as positive evidence of sexual maturity in the male rat, the results in our study were so variable that this method was discarded. Instead the procedure followed throughout this experiment was to kill the male rats at various ages and examine their testes histologically for mature spermatozoa. Fixation with

Formal-Zenker's and final staining with hematoxylin and eosin were employed with all tissues.

RESULTS. In table 1 are shown the results of feeding different amounts of desiccated thyroid upon the sexual maturity of female albino rats. In the series of 103 female controls pubescence occurred on the average in 75.4 days. The range of variability was from 45 to 132 days, but more than one-half of these rats matured between 60 and 90 days. With the smaller doses of thyroid a slight acceleration of pubescence was observed. In 21 rats which received 0.5 mgm. of thyroid daily the average age of sexual maturity was 72.9 days or 2.5 days earlier than the controls. In 17 rats which received 0.75 mgm. of desiccated thyroid daily the average age of sexual maturity was 71.7 days or 3.7 days earlier than the controls. In 22 female rats fed 1 mgm. of desiccated thyroid daily sexual maturity

TABLE 1

Summary of results of feeding desiccated thyroid on the sexual maturation of the female albino rats

DOSE OF THYROID	NUMBER OF ANIMALS	SEXUAL MATURATION		
		Maximum	Minimum	Average
mgm.	*rats*	*days*	*days*	*days*
0	103	132	45	75.4
0.5	21	102	51	72.9
0.75	17	118	41	71.7
1.0	22	105	59	73.9
5.0	10	152	57	81.6
6.0	24	113	45	93
7.50	7	130	53	93.4

occurred on the average in 73.9 days or 1.5 days earlier than the controls. On the other hand pubescence was retarded in female rats fed the higher doses of thyroid. Thus ten rats which received 5 mgm. of desiccated thyroid daily matured on the average in 81.6 days or 6.2 days later than the controls. In 24 rats fed 6 mgm. of desiccated thyroid daily and 7 rats fed 7.5 mgm. of thyroid daily pubescence was observed on the average in 93 days or 17.6 days later than the controls.

The ovaries of 40 rats (controls and thyroid fed) were removed at sexual maturity and examined histologically. They were compared with the ovaries of ten immature rats. The only striking difference between the ovary of the sexually mature and the immature female rat was the presence of the corpus luteum in the former. No histological changes could be seen in the ovaries of rats fed the lower doses of thyroid when compared with the controls. In the ovaries of rats which received 6, 7.5 or 10 mgm. of desiccated thyroid slight degenerative changes of the follicles could be seen.

Also there were fewer primary follicles than in either the controls or low thyroid rats.

The testes of 65 male rats were examined histologically. Twenty-five of these rats were controls which had never received thyroid. The earliest evidence of the production of mature spermatozoa in the controls was noted at the age of 42 days. In all except 3 cases the thyroid-fed males either did not mature as early as the controls, or if mature sperm appeared practically at the same time the thyroid-fed rats showed a smaller quantity of mature spermatozoa than the controls. The three exceptions are as follows: one rat fed 10 mgm. of thyroid daily which died at 40 days showed many adult spermatozoa. Another male rat fed 10 mgm. of thyroid daily showed as advanced maturation of the spermatozoa at 65 days as the

TABLE 2

Summary of effect of feeding desiccated thyroid upon the weight and testes of the male albino rat

DOSE OF THYROID	NUMBER OF ANIMALS	PER CENT DIFFERENCE IN WEIGHT (COMPARED WITH CONTROLS)					
		Body weight			Weight of testes		
		Maximum	Minimum	Average	Maximum	Minimum	Average
mgm.							
0.5	3	+6.02	−20.0	−4.16	+27.1	−27.0	−3.5
0.75	5	+8.3	−5	+3.9	−20.7	−34.7	−25.8
1.0	9	+18.8	−23.2	−0.2	+4.4	−39.5	−20.2
2.0	4	+15	−20	−2.1	−14.5	−24.3	−21.5
4.0	6	+25.7	−33.3	−4.01	−14.2	−39.4	−27.8
5.0	3	+13.8	−32	−2.2	−13.1	−50.9	−25.5
6.0	5	+18	−24.6	−10.5	−20.8	−32.8	−26.3
10.0	5	+21	−10.1	+7.1	+24.3	−56.6	−11.7

controls of the same age. A third male rat which received 5 mgm. of desiccated thyroid daily showed ripe spermatozoa in contradistinction to the control, where no adult spermatozoa could be seen.

Another constant finding was a marked diminution in the relative weight of the testes of the thyroid-fed rats compared with the controls. The average weight of the testes of the 40 thyroid-fed rats was 20.3 per cent less than the 25 controls. The details will be seen in table 2, where, also, the effect of thyroid upon body weight is shown. Although the body weight of the male rats fed thyroid was increased in approximately as many cases as it was decreased, an average loss in body weight of 1.51 per cent was found. Even in the rats whose body weight increased on thyroid feeding compared with the controls the testes showed a definite loss in weight except in 4 cases. One of these was the male fed 10 mgm. of thyroid mentioned above in which mature spermatozoa were observed at 40 days. The body weight

of this rat exceeded that of the control by 10.5 per cent and the testes exceeded those of the control by 24.3 per cent. A litter mate of the foregoing male also, receiving 10 mgm. of desiccated thyroid daily, gained 21 per cent in body weight and the testes gained 19 per cent. In a third exception receiving 0.5 mgm. of thyroid daily the body weight exceeded that of the control by 6.02 per cent and the testes exceeded those of the control by 27.1 per cent. A fourth male of this group fed 1.0 mgm. of thyroid daily exceeded the body weight of the control by 12.9 per cent and the testes were 4.4 per cent heavier than the control. In the second and fourth cases the increase in the weight of the testes was not as great as that of the body weight; therefore there was even a relative loss in the weight of the testes among the exceptions.

Discussion. The depressing influence of thyroid feeding upon the gonads of the male rat observed in this study agrees with the findings of Zawodowsky (18) on roosters and Dulzetto (29) on rats. The three apparent exceptions in our experiment could only be significant if a larger series were studied. The observations on the normal male rat in the present study correspond to the histological findings of Allen (27) who noted ripe spermatozoa at 37 to 40 days (compared to our 40–42 days). But, as Stone (30) points out, although adult spermatozoa may be found in the testes between 40 and 45 days, actual copulation does not usually occur until about 65 days. For Stone showed that the organs of intro-mission are not developed sufficiently to allow successful impregnation of the female before this age is reached.

The difference between the results on the female rats in our study and the results of others may be due to the difference in the dose of thyroid. For it is only on the smaller doses of thyroid that we found pubescence slightly accelerated. The reverse was true at higher doses. Thus the depressing effect on the activity of the rat measured by the revolving cage by Wang (21) can be explained upon the basis of his dose of 2, 4 or 8 grains or 120, 240 and 320 mgm. of thyroid per 100 grams of food. In our study we fed doses of desiccated thyroid ranging only from 5 to 100 mgm. per 100 grams of food. In Dulzetto's study "Endothyroidein" and not desiccated thyroid was administered and a comparison of his results with ours is, therefore, difficult. However, considering the severe follicular degeneration of the ovary which Dulzetto describes we may conclude that his results were similar to ours with high doses of desiccated thyroid. The figures for sexual maturity in the normal albino female rat in this study agree with those of Donaldson (26) who reports pubescence at approximately 80 days and Slonaker who found 80.5 days.

CONCLUSIONS

1. Female albino rats fed 0.5 to 1 mgm. of desiccated thyroid daily show a slight acceleration in sexual maturation, while those fed 5 to 10 mgm. of thyroid daily show a definite retardation in pubescence.

2. In male albino rats fed 0.5 to 10 mgm. of desiccated thyroid daily a decidedly lower relative weight of the testes than in the controls was the rule. This relationship was seen even when the body weight of the thyroid-fed rats was greater than that of the controls.

3. In most cases the sexual maturation of the gonads of the male rat was depressed by thyroid feeding (0.5–10 mgm. per day).

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THE NERVE PLEXUS BETWEEN THE AORTA AND PULMONARY ARTERY

I. Observations on Its Nature and Function[1]

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It has been known for some time that a nerve plexus exists around the proximal portion of the ascending aorta, particularly in the region between the ascending aorta and the pulmonary artery. It has been shown, also, that this resembles histologically the carotid sinus at the bifurcations of the carotid arteries (cf. Koch, 1931). This we have been able to confirm in routine examination of human and dog material. In addition to fat tissue, connective tissue, nerve fibers, and a large number of sympathetic ganglion cells, we have found accumulations of other cells arranged in small groups. These cells were large, revealing a lightly stained cytoplasm and a small, somewhat vesicular nucleus. The groups of cells were separated from one another by connective tissue fibers which contained many small sized blood vessels. By the use of chromic stains these cells showed chromophilic properties. Often only one group of these cells was noted, but larger accumulations of these cells forming several groups were also observed. Sympathetic ganglion cells and nerve fibers were found often within or very close to these cell groups. According to their morphology and staining quality these cells must be classified as chromaffin cells.

The presence of chromaffin tissues in the region between the most proximal portion of the aorta and the pulmonary artery was noted by other observers. Trinci (1911) found them in mammals and reptiles, and Busacchi (1912–13) in the human. Maximow (1930) also mentions their occurrence. Their existence, however, is apparently not generally recognized. Thus, Kraus (1924) recently stated that with the exception of the carotid bodies, no paraganglia can be found above the aorta after birth. A close relation of chromaffin cells and ganglion cells is not unusual and has been shown to occur (Kohn, 1902; Keibel and Mall, 1912). The

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functional significance to be attached to the presence of the chromaffin tissue in this or any nerve plexus is unknown.

There is evidence that the root of the aorta has afferent nerve fibers connected with it which reflexly tend to lower the blood pressure and slow the heart, but much remains unknown as to the pathways which these fibers take. In many species these afferent fibers go up a special nerve trunk, the depressor, or as it has been recently called, the aortic nerve. The evidence for the existence of this afferent pathway is based on the following types of investigation: 1, noting by anatomic determinations the paths from the plexus (Wooldridge, 1883; Kazem-Beck, 1888; Schumacher, 1902; Köster and Tschermak, 1902; Dogiel, 1911; Tello, 1924, and others); 2, noting a more or less transient rise in blood pressure and increase in heart rate on cutting the nerves (Kisch and Sakai, 1923; Hirsch and Stadler, 1904; Hering, 1927; Koch, 1930; Bayliss, 1893; Cyon and Ludwig, 1866; Sewall and Steiner, 1885); 3, noting that stimulation of the central end of the nerve causes a fall in blood pressure and a drop in heart rate (Sollmann and Pilcher, 1912; Cyon and Ludwig, 1866; Bayliss, 1893; Hering, 1927; Langley, 1912; Porter, 1914, Jonnesco and Ionescu, 1926); 4, obtaining action currents in the nerve with each heart cycle (Einthoven, 1908; Adrian, 1926; Bronk, 1931; Rijlant, 1932), or by distention of aorta by mechanical means or by a rise of blood pressure (Köster and Tschermak, 1903; Osborne, 1924); 5, obtaining a depressor response of the heart and blood pressure by distention of the intact or isolated aorta (Köster and Tschermak, 1903; Eyster and Hooker, 1908; Daly and Verney, 1926, 1927; Heymans and Landon, 1925; Anrep and Segall, 1926; Sollmann and Pilcher, 1913).

The case for the aortic nerves, therefore, seems complete. But with the discovery of the carotid sinus and the establishment of its rôle, chiefly as the result of the work of Hering (1927), Heymans (1929), Koch (1931), which is summarized in their reviews, the attention has been somewhat drawn away from the aortic nerves.

While the depressor action of the aortic nerve is established, it must be borne in mind that evidence exists that pressor reflexes also can be propagated in the nerves from the heart and blood vessels. Thus Bochefontaine and Bourceret (1877) found that stimulation of the pericardium of the heart caused a reflex pressor response. Francois-Franck (1899) found that in the cervical sympathetic there were both pressor and depressor fibers. This was confirmed by Danielopolu (1927). Francois-Franck (1878–1879) also demonstrated that the vagi had both pressor and depressor fibers, which was confirmed by Langley (1912) and many others. It has been shown by Weber (1908), among others, that a change in current strength will alter a pressor into a depressor reflex or vice versa. Hürthle (1889) and Velluda (1927) have shown that stimulating one part of the

vagus will give a depressor response in the dog, and stimulating another part will give a pressor response.

It was decided that more direct information concerning the rôle of this plexus between the aorta and pulmonary artery might be obtained, as far as the effect on blood pressure and heart rate was concerned, by stimulating this plexus electrically and noting the result. The effect of severing the various possible afferent pathways was studied also, with the hope that the path of these various types of fibers might be mapped out.

The reflex effect of stimulation of this region was studied and the pathways taken were analyzed in fifteen dogs anesthetized with morphine and sodium barbital.

METHOD. The effects on the heart rate and blood pressure of the femoral artery were analyzed. The dog's chest was opened and artificial respiration instituted. The pericardium was split to expose the base of the heart. The fat around the aorta was dissected, exposing the region beneath the ascending aorta and arch adjacent to the pulmonary artery. The region exposed was stimulated with a faradic current using platinum electrodes; a signal magnet in series showed the time of onset and offset of the electrical stimulation. Many regions of the exposed surface of the aorta and pulmonary artery were explored with the stimulating electrode. Occasionally a vagus slowing due to stimulation of efferent vagus fibers was found, in which case this complicating effect was avoided by injecting atropin intravenously. In most of the experiments the vagi were then cut low in the neck and the stimulation of the region repeated. In five animals this was followed by complete denervation of the heart, after which the region of the plexus was again stimulated. In three experiments instead of denervation, the plexus region was cauterized with carbolic acid and alcohol and the stimulation repeated. Care was taken in all experiments to avoid several artifacts which can modify the blood pressure, viz., 1, spread of stimulating current to the phrenic nerves, which by stimulating the diaphragm was found to cause, mechanically, a rise in blood pressure; 2, partial occlusion of the pulmonary artery which leads mechanically to a blood pressure drop if care is not taken in the application of the stimulating electrodes.

RESULTS. It was found that stimulation of the exposed parts of the aorta and pulmonary artery, except in the region of the plexus, is without effect on the blood pressure and heart rate. The results of stimulating the plexus are summarized in table 1. It will be seen that in only four out of fifteen experiments was a slight depressor effect found—in the other eleven a noticeable pressor effect was obtained. The changes in blood pressure occurred after a variable latent period and outlasted the end of stimulation in every instance. There was no correlation between the direction and amount of change of the blood pressure and the initial blood pressure level.

The heart rate change was variable. In eight of the fifteen animals no change occurred; in one there was slowing, in the others an acceleration. The onset of the heart rate change occurred after a latent period different from that for the blood pressure change—usually the former period was longer.

Cautery abolished the reflex in the three experiments in which it was employed. In only two of the ten experiments did vagus section abolish the reflex response. In five the response was decreased, in one a depressor response was changed to a pressor. In two experiments no change in the

TABLE 1

Effect of stimulating the aortic-pulmonary plexus under different conditions

EXPERIMENT NUMBER	INITIAL MEAN BLOOD PRESSURE	EFFECT ON BLOOD PRESSURE OF STIMULATING AORTIC PULMONARY PLEXUS	EFFECT ON HEART RATE OF STIMULATING AORTIC PULMONARY PLEXUS	DOES CHANGE PERSIST AFTER VAGI ARE CUT?	DOES CHANGE PERSIST AFTER COMPLETE DENERVATION?	DOES CHANGE PERSIST AFTER CAUTERIZATION?
	mm. Hg	*mm.* Hg				
KS 2	80	+30	0*			
KS 4	135	−10	0			
KS 5	80	−10	−	Yes (less)		
KS 6	85	+15	0	Yes		
KS 7	90	+10 to +20	+	Yes (less)	No	
KS 8	85	+20	+	No		
KS 9	120	+20	0	No		
KS 10	80	−20	0	Changes to a pressor effect	No	
KS 11	90	−10	0	Yes	No	
KS 12	80	+30	0			
KS 13	60	+40	+	Yes (less)		No
KS 14	100	+50	+			No
KS 15	80(?)	+40	0	Yes (less)	No	
KS 16	80	+35	+ (slight)	Yes (less)	No	
KS 17	130	+30 to +40	+ (slight)			No

* 0 means no change; − a decrease, and + an increase in heart rate.

response was noted. However, in every one of the five trials complete denervation abolished the response.

Discussion. These experiments show that pressor and depressor fibers are to be found in this aortic-pulmonary plexus and that their pathway is not only via the vagus nerve but also by way of the sympathetic chain. These experiments cannot determine whether these fibers come only from the aorta or in part also from the heart. The concentration of these afferent fibers in this plexus forms a sort of "internal capsule" which may be important for other types of afferent fibers such as those transmitting pain.

SUMMARY

1. A nerve plexus rich in ganglion cells and chromaffin cells exists between the ascending aorta and the pulmonary artery.

2. Both pressor and depressor fibers can be demonstrated in this plexus; the former predominating in our series of experiments.

3. These afferent fibers run not only via the vagi but also by way of the sympathetic chain.

4. The concentration of these afferent fibers in this plexus forms a sort of "internal capsule" which may be important for other types of afferent fibers such as those carrying pain sensations.

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